UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

6 September 2007

MEMORANDUM

Subject:	Name of Pesticide Product:	ADVANTAGE PLUS 9 FOR CATS
	EPA Reg. No. /File Symbol:	11556-REA
	DP Barcode:	D338710
	Decision No.:	215319
	PC Codes:	129099 [Imidacloprid: 9.1%]
		129032 [Pyriroxyfen: 0.46%]

From: Byron T. Backus, Ph.D., Toxicologist
Technical Review Branch
Registration Division (7505P)

Byron T. Backus
9/6/2007

To: Kable Davis/Venus Eagle, RM 01
Insecticide-Rodenticide Branch
Registration Division (7505P)

M Hasha
9/6/07

Registrant: BAYER HEALTHCARE LLC

FORMULATION FROM LABEL:

Active Ingredient(s):	% by wt.
129099 Imidacloprid	9.1%
129032 Pyriproxyfen	0.46%
Inert Ingredient(s):	90.44%
Total:	100.00%

ACTION REQUESTED: The Risk Manager requests:

Please review the attached domestic animal safety studies to determine if they support product registration. I have also included copies of the cover letter, amended label and CSF…"

BACKGROUND:

The material received for review includes three companion animal (each with 8-week old kittens) studies. One study (MRID 47089401, with additional information in MRID 47089402) involved testing two groups of kittens; one group received 5X of a formulation containing the active ingredients (9.1% Imidacloprid and 0.9% Pyriproxyfen) on Days 0, 7, 14 and 21, while the second group was dosed with ~5.6X the solvents and inerts (no actives) of the proposed formulation on the same days. In the second study (MRID 47089403, with additional information in MRID 47089404) there were again two groups of kittens; one group received 5X applications of the solvents and inerts on Days 0, 7, 14 and 21, while the second group was untreated. In the third study (MRID 47089405, with additional information in MRID 47089406) there were two groups of kittens; one group received 3X applications of the solvents and inerts on Days 0, 7, 14 and 21, while the second group was untreated.

RECOMMENDATIONS:

1. Each of the three studies has been classified as supplementary data. An adequate (5X) margin of safety associated with the use exposure to the solvents and/or inerts of this formulation has not been demonstrated. Until it is shown that there is an acceptable 5X margin of safety, these studies cannot be used, by themselves, to support the use of this and/or similar formulations on 8-week-old kittens.

> A. In the study MRID 47089401 two of the 14 kittens dosed with ~5.6X the solvents and inerts (no actives) of the proposed formulation had to be euthanized on Day 1. In the study MRID 47089403 two of the 16 kittens dosed with 5X the solvents and inerts (no actives) of the proposed formulation had to be euthanized on Day 1. All four of the euthanized kittens showed clinical signs which included tremors, with incoordination and/or depression and/or dilated pupils and/or rapid breathing. At gross necropsy, each of the four kittens showed a distended urinary bladder. Microscopic examination of all four showed necrosis of cells in the external granular layer of the cerebellum.
>
> B. All four of the kittens which were euthanized in these two studies showed hematology and clinical chemistry findings on Day 1 which included increased neutrophil counts, decreased calcium and phosphorus levels and increases in alkaline phosphatase, aspartate animnotransferase and alanine aminotransferase.
>
> C. Some additional kittens in these first two studies also showed clinical signs (tremors, etc.) of toxicity following an exposure to the test material, but subsequently recovered. Hematology and blood chemistry findings showed some changes similar to (but not as pronounced) as those observed in the euthanized kittens.

2. In the study MRID 47089405 one of the 14 kittens dosed with 3X the solvents and inerts of the proposed formulation showed symptoms (tremors, incoordination) in the period from Day 1 to 3 following application on Day 0. This kitten also had hematology and blood chemistry findings similar to (but not as pronounced) as those observed in the euthanized kittens. The occurrence of these clinical signs of toxicity indicates that the margin of safety associated with exposure to the solvents/inerts of this formulation is less than 3X.

3. No significant indications of toxicity were observed in Group A (exposed to the formulation with active ingredients) kittens of the study in MRID 47089401. One possible explanation for the greater severity of symptoms in kittens exposed to the solvents/inerts was that the observed toxicity was due to oral ingestion of the solvent(s), but that a bad taste or some other unpleasant sensation association with the active ingredients resulted in considerable less oral ingestion by kittens that were exposed to the formulation with actives.

4. TRB is aware that the percentages of solvents and inerts of this proposed formulation are similar to those of several existing imidacloprid-containing products which are registered for use on kittens of 8 weeks of age and older. A check of our records indicates the supporting kitten study (dated August 30, 1996, in MRID 44157302) for a representative product was reviewed and accepted by HED 9/17/97, but that the study used two groups, one of which was treated with the 9.1% imidacloprid at 5X (2.0 mL) the recommended use rate of 0.4 mL at weekly intervals for 8 treatments, while the second was treated with the vehicle control at the recommended use (exposure) rate of 0.4 mL at weekly intervals for 8 treatments. This study was conducted prior to the publication (August, 1998) of the 870.7200 companion animal safety study guidelines which state that: "The vehicle control should be administered at a 5X level. The vehicle should contain the inert ingredients at the maximum levels that would appear in the 5X formulation."

5. The studies (MRIDs 47089401 through 47089406) have laboratory dates of 2000-2001, but have only been recently submitted to the Agency. Since these studies demonstrate potential adverse [6(a)(2)] effects associated with exposure to the solvent(s)/inerts in this formulation, they should have been submitted to the Agency as soon as the findings became known.

EPA Primary Reviewer: Byron T. Backus, Ph.D.
Technical Review Branch, Registration Division (7505P)
EPA Secondary Reviewer: Masih Hashim, D.V.M., Ph.D.
Technical Review Branch, Registration Division (7505P)

Signature: Byron T. Backus
Date: 9/6/2007
Signature: M. Hashim
Date: 9/6/07

DATA EVALUATION RECORD

STUDY TYPE: Companion Animal Safety - Kittens (OPPTS 870.7200)

PC CODES: 129099 (Imidacloprid); 129032 (Pyriproxyfen)

DP BARCODE: D338710

DECISION NO.: 215319

RISK MANAGER: (EPA): 01

TEST MATERIAL AND PRODUCT: Imidacloprid (9.1%) and Pyriproxyfen (0.9%) in the final formulation. After examinining page 2 of the Confidential Appendices in MRID 47089401 and comparing the information with that in the CSF (dated March 8, 2007) for 11556-REA (Advantage® Plus 9 for Cats, with 9.1% Imidacloprid and 0.46% Pyriproxyfen) it is concluded that studies conducted on the test formulation could be used to support the registration of 11556-REA provided they demonstrate an adequate margin of safety.

CITATION: Abraham, A. S. (2001). Evaluation of the General Safety of 9.1% Imidacloprid with 0.9% Pyriproxyfen Spot-on Formulation in the Target Species, 8-Week Old Kittens. Performing Laboratory: Intervet Inc. (formerly a facility owned and operated by Bayer Corporation Agriculture Division Animal Health), DeSoto Research Facility, DeSoto, Kansas 66018. Laboratory Project ID 75120 (150.851). Study Dated June 8, 2001. MRID 47089401. 160 p. + a 2 p. confidential appendix.

SPONSOR: Bayer Corporation Agriculture Division

SUBMITTER: Bayer HealthCare LLC, Animal Health Division, P.O. Box 390, Shawnee, KS 66201

EXECUTIVE SUMMARY: In a companion animal safety study (MRID 47089401), there were two groups, each containing 7 male and 7 female kittens (from 7 weeks 5 days to 8 weeks old; day -1 bodyweights: males: 1.14-2.09 lbs; females: 1.42-2.10 lbs; source: Harlan Sprague Dawley, Inc., Madison, WI). Kittens in Group A were treated with the formulation containing actives (Imidacloprid: 9.1%; Pyriproxyfen: 0.9%) at 5X the label-specified use application rate of 0.4 mL (5 x 0.4 mL = 2.0 mL) while kittens in Group B were treated with 2.0 mL of vehicle (solvent) material (equal to ~5.6X the amount of solvents and non-active ingredients from a single use application of the proposed product). The dose was applied topically on the backside of the head and the neck of each kitten to avoid runoff. Kittens were treated on Days 0, 7, 14 and 21; the proposed label indicates once-a-month treatment, so that each of the kittens in Group A received a cumulative total of 20X of the proposed monthly dosage of the formulation while kittens in Group B received a cumulative total of ~22.4X of the proposed monthly dosage of solvents and non-active ingredients. At the end of the study the heaviest kitten was 3.93 lbs, so none reached a weight >9 lbs (4.1 kg) which would have resulted in an increase in the 1X dose from 0.4 to 0.8 mL.

On the days of dosing (Days 0, 7, 14 and 21) each kitten was observed five times, once prior to dosage and then at hourly intervals for four hours after application. Otherwise, clinical observations were made twice a day. Individual daily food consumption was determined visually, using a scoring

system (≥75% consumption = 1, 25-75% consumption = 2, ≤25% consumption = 3). The kittens were weighed at six times before and during the study (Days -14, -7, -1, 13, 28 and 38). Blood samples were taken on Days -7, -1, 1, 22, and 38. Blood samples from four kittens were also collected on study day 2, from 3 kittens on study days 22-23 (insufficient initial samples and machine malfunction) and blood was collected a second time from one kitten on day 38 (due to clotting). Prothrombin time and activated partial thromboplastic time measurements were not done because of the comparatively large amount of blood required for these tests and the age of the kittens; this protocol deviation had been previously discussed with and accepted by the Agency.

Signs of toxicity in Group A (formulation with active ingredients) kittens following the Day 0 treatment were salivation (2 females) and salivation accompanied by sneezing (one male). One of the two affected females had nasal discharge on Day 1. Following treatment on Day 7 one male (#792) showed depression, rapid respiration and vomiting; however, this male had also shown these signs shortly before dosage. Male #792 subsequently showed rapid respiration, slowness of movement and unsteadiness on Day 8, and received 24 mL Lactated Ringers Solution (LRS) + 2 mg Ceftiofur + 0.5 mg Re-Covr. Following the Day 7 treatment another male (#775) was slow to move and depressed, and also had diarrhea on Days 8 and/or 9. On Day 9 #775 was treated with 24 mL LRS + 2 mg Ceftiofur + 0.5 mg Re-Covr, also 2 mg Ceftiofur on Day 10. It is stated that symptoms in #775 were not due to test material and may have been from bacterial enteritis. During the observation period following the Day 14 treatment 2 females showed pruritis.

Two male kittens (same sire, but from different mothers) in Group B had adverse reactions to the solvent control (vehicle) and died on Day 1. Signs observed prior to death in one of the kittens were sneezing, salivation, dilated pupils, rapid breathing, generalized tremors and slow movement. Signs in the other kitten were dilated pupils, rapid breathing and generalized tremors. In the blood samples taken on day 1 both of these male kittens showed a number of physiologically significant hematology and blood chemistry changes, including increases in neutrophils, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP), and decreases in potassium (K), phosphorus (P) and calcium (Ca). At gross necropsy both had distended urinary bladders. On microscopic examination, both showed necrosis in the external granular layer of the cerebellum and lymphoid tissues. Other necropsy findings were incidental and/or considered to be secondary to the moribund condition of the kittens.

There were no signs of toxicity in other Group B kittens following the Day 0 treatment. Following the Day 7 treatment one Group B male had a number of signs which included tremors, slowness of movement, vomiting and dilated pupils on Days 8-9. The same male also had sneezing following the application on Day 14, followed by depression, tremors, disorientation and dilated pupils on Days 16-18. This male (#776) had a different sire from that of the two kittens which died. One female showed pruritis in the observation period following the Day 14 treatment.

One female (#788) in Group B had tremors on days 22 and 23 and dilated pupils, unsteadiness and apprehensiveness on day 23, and also had noticeably reduced potassium, phosphorus and calcium levels on Day 22. In addition, female solvent control kitten #797 (not reported as having any signs of toxicity) had slightly reduced potassium and somewhat elevated ASP and ALT on Day 22. Values were within normal ranges for both of these kittens on Day 38. One possible explanation for the greater severity of symptoms seen in the Group B (solvent control) kittens was that the toxicity was a result of oral ingestion of the solvent(s), but that a bad taste or some other unpleasant sensation associated with the active ingredients resulted in considerably less oral ingestion of the test material in the Group A kittens.

This study is classified as **Supplementary** as a companion animal safety study (OPPTS 870.7200), as it does not demonstrate a 5X margin of safety associated with exposure to the solvents/inerts and the proposed use of the formulation (with actives) in 8 week old kittens.

COMPLIANCE: Signed and dated Quality Assurance (p. 4), [No] Data Confidentiality (p. 2) and Good Laboratory Practice Compliance (p. 3) statements are present.

I. MATERIAL

MATERIALS

1. Test material: 9.1% Imidacloprid with 0.9% Pyriproxyfen (w/w) spot-on formulation.
   Description: A liquid with a specific gravity of (see p. 156 of MRID 47089401) 1.097 g/mL.
   Lot No.: 99-901-66.
   Storage: Stored in amber glass bottles at room temperature.
   Placebo: The test material without the two active ingredients.
   Description: A liquid with a specific gravity of (from p. 157 of MRID 47089401) 1.0674 g/mL.
   Lot No.: 99-901-68
   Storage: Stored in amber glass bottles at room temperature (15° -30°C)

2. Administration: Topically applied to the backside of the head and the neck of each kitten to avoid run off.

3. Test animals
   Species: Cat
   Breed: Domestic Short hair
   Ages and weights at study initiation (Day 0, day of dosing): Males: 7 weeks 5 days to 8 weeks; 1.14 to 2.09 lbs; Females: 7 weeks 6 days to 8 weeks; 1.42 to 2.10 lbs. [Note: weights for both sexes are for Day -1].
   Source: Harlan Sprague Dawley, Inc., Madison, WI 53744
   Vaccinations: The kittens had been vaccinated with a four way feline vaccine, Fel-O-Vax IV (Feline Rhinotracheitis, Calici, Panleukopenia, Chlamydia Psittaci Vaccine) prior to acclimation.
   Housing: individual in cages with at least 3 ft^2 of floor space and at least 24 inches high.
   Diet: Harlan Teklad® (commercial dry cat feed) and a canned kitten food (Feline Growth) from Hill's Pet Nutrition, Kansas City, MO. (fed once daily, however, no information is provided as to the amount that was offered).
   Water: Tap water, *ad libitum*
   Environmental conditions:
   Temperature: (not stated)
   Humidity: (not stated)
   Air changes: (not stated)
   Photoperiod: 9-14 hours of lighting/day
   Acclimation period: 14 days

II. STUDY DESIGN

A. IN LIFE DATES

From the report (p. 12 of MRID 47089401): Day 0 was September 21, 1999. The experimental phase of the study was completed on February 28, 2000 (histopathology slides read).

B. ANIMAL ASSIGNMENT/ DOSAGE AND ADMINISTRATION

From p. 14 of MRID 47089401: "Twenty-eight animals were randomly allocated to two groups. Animals were blocked by sex and ranked by ascending order of study day -1 body weight and assigned a random number. From the first block (female), the animal with the larger of the first two random numbers was assigned to Group A (test substance), and the smaller to Group B (placebo) and so forth until all the animals of the same sex were assigned. This procedure was repeated for the males..."

From p. 19 of MRID 47089401: "Seven males and seven females in group A were dosed weekly for four weeks with 5 times the monthly use rate volume (2 mL [5 x 0.4 mL dosage rate for kittens weighing less than 9 lbs]...) of test substance. This resulted in a 20X the monthly use volume applied during a month's time."

"Seven male and seven females in group B were dosed with 5 times the monthly use rate volume (2 mL for kittens up to 9 lbs of body weight) of placebo without either of the active ingredients. This treatment resulted in 5.6X the vehicle content, as the test article was 10% by weight (11% by volume) of active ingredients and 0.2 mL of active ingredients. Thus, by giving the kitten a full 2 mL of vehicle (instead of 1.8 mL of vehicle), the kitten received a 5.6X overdose of vehicle. This resulted in a 22.4X the monthly use volume applied in a month's time."

From p. 20 of MRID 47089401: "The dose was administered topically on the backside of the head and the neck of each kitten to avoid run off of the test or control substance... The kittens were dosed four times: on study days 0, 7, 14, and 21."

TABLE 1. Study design

Group & Weight Range (lb)		Number of kittens	Mean Kitten Weight			
			Mean Kitten Wt ± S.D. (lb) on Day -1 (before 1st application)	Mean Kitten Wt ± S.D. (lb) on Day 13 (before 3rd application)	Mean Kitten Wt ± S.D. (lb) on Study Day 28	Mean Kitten Wt ± S.D. (lb) on Study Day 38
Con-trol (B)	males ≤ 9 lb	7*	1.62 ± 0.28	2.18 ± 0.31	3.01 ± 0.36	3.36 ± 0.35
	females ≤ 9 lb	7	1.73 ± 0.21	2.26 ± 0.19	2.90 ± 0.21	3.25 ± 0.26
	combined ≤ 9 lb	14**	1.67 ± 0.24	2.23 ± 0.24	2.94 ± 0.27	3.29 ± 0.29
5X (A)	males ≤ 9 lb	7	1.60 ± 0.30	2.06 ± 0.18	2.98 ± 0.32	3.36 ± 0.31
	females ≤ 9 lb	7	1.75 ± 0.17	2.24 ± 0.22	3.04 ± 0.32	3.36 ± 0.33
	combined ≤ 9 lb	14	1.68 ± 0.25	2.15 ± 0.22	3.01 ± 0.31	3.36 ± 0.31

Data calculated from information on p. 42 of MRID 47089401.
*5 kittens on Days 13, 28 and 38, as two kittens had died on Day 1.
**12 kittens on Days 13, 28 and 38, as two kittens had died on Day 1.

C. DOSE SELECTION RATIONALE

From p. 11 of MRID 47089401: "The study was conducted to evaluate the safety of 9.1% Imidacloprid with 0.9% Pyriproxyfen (w/w) spot-on formulation or vehicle in 8-week old kittens applied at 5 and 5.6X times the use volume rate, respectively, at one week intervals for a total of 4 weeks. This study was designed as a limit test, and a full study

with three dose levels 1X, 3X and 5X was not conducted. This study supports the registration of other similar formulations."

D. EXPERIMENTAL DESIGN

There were two groups, each containing 7 male and 7 female kittens (from 7 weeks 5 days to 8 weeks old; day -1 bodyweights: males: 1.14-2.09 lbs; females: 1.42-2.10 lbs). Kittens in Group A were treated with the proposed product at 5X the label-specified use application rate of 0.4 mL (5 x 0.4 mL = 2.0 mL) while kittens in Group B were treated with 2.0 mL of vehicle (equal to ~5.6X the amount of solvents and non-active ingredients from a single use application of the proposed product). The dose was applied topically on the backside of the head and the neck of each kitten to avoid runoff. The dose was administered by parting the hair and using a syringe without a needle. Kittens were treated on Days 0, 7, 14 and 21; since the proposed label indicates once-a-month treatment, each of the kittens in Group A received a cumulative total of 20X of the proposed monthly dosage of the formulation while kittens in Group B received a cumulative total of ~22.4X of the proposed monthly dosage of solvents and non-active ingredients. At the end of the study the heaviest kitten was 3.93 lbs, so none had reached a weight >9 lbs (4.1 kg) which would have resulted in an increase in the 5X dose from 2.0 to 4.0 mL.

On the days of dosing (Days 0, 7, 14 and 21) each kitten was observed five times, once prior to dosage and then at hourly intervals for four hours after application. Otherwise, clinical observations were made twice (once in the a.m., once in the p.m.) a day. Individual daily food consumption was determined visually, using a scoring system ($\geq$75% consumption = 1, 25-75% consumption = 2, $\leq$25% consumption = 3). The kittens were weighed at six times before and during the study (Days -14, -7, -1, 13, 28 and 38). Blood samples were taken on Days -7, -1, 1, 22, and 38. Blood samples from four kittens were also collected on study day 2, from 3 kittens on study days 22-23 (insufficient initial samples and machine malfunction) and blood was collected a second time from one kitten on day 38 (due to clotting). Prothrombin time and activated partial thromboplastic time measurements were not done because of the comparatively large amount of blood required for these tests and the age of the kittens; this protocol deviation had been previously discussed with and accepted by the Agency.

"Physical examinations were performed on the kittens on study day -14, on study day -1, and on study day 35."

E. CLINICAL PATHOLOGY PARAMETERS

Blood samples were collected from each kitten on study Days -7, -1, 1, 22 and 38. There is no indication within the report of fasting prior to collection of blood. The CHECKED (X) parameters were examined:

a. Hematology

X		X	
X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Absolute and percent basophil count
		X	Absolute and percent eosinophil count
X	Leukocyte count (WBC)*	X	Absolute and percent lymphocyte count
X	Erythrocyte count (RBC)*	X	Absolute and percent monocyte count
		X	Absolute and percent neutrophil count
X	Platelet count (PLTS)	X	Mean corpuscular HGB (MCH)*
	Blood clotting measurements	X	Mean corpusc. HGB conc.(MCHC)*
	(Thromboplastin time)	X	Mean corpusc. volume (MCV)*
	(Clotting time)		
	(Prothrombin time [PT])*		
	(Activated partial thromboplastin time [APTT])*		

*Recommended in OPPTS 870.7200 Guidelines. The Prothrombin time and Activated partial thromoplastin time were not done because of the comparatively large volume of blood required for these tests and the age of the kittens. This deviation had been accepted by the Agency prior to initiation of this study.

b. Clinical chemistry

X	ELECTROLYTES	X	OTHER
X	Calcium*	X	Albumin (Alb)*
X	Chloride*	X	Creatinine (Crea)*
	Magnesium	X	Blood urea nitrogen (BUN)*
X	Phosphorus*		Total Cholesterol
X	Potassium*	X	Globulin (Glob)*
X	Sodium*	X	Glucose (Gluc)*
		X	Total bilirubin (T Bil)*
	ENZYMES	X	Direct bilirubin (D Bil)*
			Total protein (TP)*
X	Alkaline phosphatase(ALP or ALK)*		Triglycerides
	Cholinesterase(ChE)		Serum protein electrophoresis
	Creatine phosphokinase	X	Albumin/Globulin (A/G) ratio
	Lactic acid dehydrogenase(LDH)		Lipase
X	Serum alanine aminotransferase (ALT or SGPT)*		
X	Serum aspartate aminotransferase(AST or SGOT)*	X	BUN/Creatinine ratio
		X	Ca/Phos Ratio
	Gamma glutamyl transpeptidase(GGT)	X	Na/K Ratio
	Amylase		

*Recommended in OPPTS 870.7200 Guidelines.

F. CONCOMITANT MEDICATIONS

From p. 20 of MRID 47089401: "On study day -4, coccidiosis was diagnosed in the kittens. Starting on study day -4 through study day -1, the kittens were treated once daily orally with Sulfamethoxisol/Trimethoprim liquid at approximately 15 mg and 3 mg/pound body weight, respectively. Then from study day 3 through study day 9 the kittens were treated once daily orally with Albon Suspension (Sulfadimethoxine). On the first day of dosing (study day 3) the animals received approximately 25 mg of Sulfadimethoxine per pound of body weight. On subsequent days (study days 4 to 9) the animals received approximately 12.5 mg of Sulfadimethoxine per pound of body weight."

G. STATISTICS

Although means and standard deviations were calculated for some parameters (such as body weight), statistical tests were primarily applied to chemistry and hematology pathology parameters. From p. 132 of MRID 47089401: "This study is intended to confirm the general safety of the test substance and approximately the same number of adverse effects is expected between the two groups. Adverse effects will be summarized in tables. Certain types of adverse effects may be grouped together, depending on the clinical presentation, such as all effects, all transient effects or all blood chemistry effects. If the number or pattern of effects elicit clinical interest, incidence rates will also be compared between groups." For the clinical pathology parameters, it is stated (p. 22 of MRID 47089401): "For each animal and for each clinical pathology test, a baseline value was calculated by averaging the two pretreatment measurements (study days -7 and -1). Each clinical pathology test was then analyzed with a multivariate repeated measures ANOVA (baseline, study days 1, 22, and 38) including terms for Group, Sex, Animal (random), Day, and Group*Day as the predictors..."

H. DISPOSITION OF ANIMALS

From p. 14 of MRID 47089401: "One animal not included in the experimental phase of the study was euthanized due to poor health on September 21, 1999. Two animals [on test] died on study day 1. Twenty-five animals in the experimental phase of the study were euthanized on November 01, 1999. One animal in the experimental phase of the study and one animal not in the experimental phase of the study were given for adoption as pets." According to the OPPTS 870.7200 Guidelines: "Routine sacrifice or necropsy is not required for surviving animals."

I. COMPLIANCE

Signed and dated Quality Assurance [p. 4], [No] Data Confidentiality [p. 2], and Good Laboratory Practice (GLP) Compliance [p. 3] Statements were present.

III. RESULTS

A. EXPOSURE LEVELS

Refer to Table 1 of this DER. Kittens in the control group (Group B), all weighing $\leq$ 9.0 lb, were dosed with 2.0 mL of the test material formulation without active ingredients at

each application, while kittens in the test group (Group A), also all weighing ≤ 9.0 kg, were dosed with 2.0 mL of the complete formulation at each application. Applications were made on Days 0, 7, 14 and 21.

B. MORTALITY

Two control (Group B) male kittens died on Day 1; all other kittens survived the 38-day observation period.

C. CLINICAL SIGNS

Group A (test material treated): Signs of toxicity following the Day 0 treatment were salivation (2 females) and salivation accompanied by sneezing (one male). One of the two affected females had nasal discharge on Day 1. Following the Day 7 treatment one male was slow to move and depressed on Days 8-9. Another male showed rapid respiration, depression and vomiting in the 4-hour observation period following treatment, as well as unsteadiness on day 8; however, this male had shown vomiting, depression and rapid respiration immediately before dosage. Following the Day 14 treatment 2 females showed pruritis in the 4-hour observation period after dosage.

Two male kittens (same sire, but from different mothers) in Group B had adverse reactions to the control substance and died on Day 1. One of the kittens had shown sneezing and salivation in the four hour period after treatment. Both kittens showed rapid breathing, dilated pupils, and tremors on day 1 prior to death.

There were no signs of toxicity in other Group B (control) kittens following the Day 0 treatment. Following the Day 7 treatment one Group B male had a number of signs which included tremors, slowness of movement, vomiting and dilated pupils on Days 8-9. The same male also had sneezing following the application on Day 14, followed by depression, tremors, disorientation and dilated pupils on Days 16-18. This male (#776) had a different sire from that of the two kittens which died. One female showed pruritis in the observation period following the Day 14 treatment.

TABLE 2a. Observed Signs of Toxicity After First Application (Day 0)

Group	Signs in the 4-hour observation period	Signs in the two days following treatment
Control (B)	Sneezing, salivation in male #783.	Rapid respiration, dilated pupils, tremors & death in 2 males (#783 & #791) on day 1. Kitten #783 received 12 mL Lactated Ringers Solution subcutaneously on day 1.
5X Test Material (A)	Sneezing in 2 females (#773, #777); salivation & sneezing in one male (#775)	Nasal discharge in one female (#777).

Data from information on p. 38 of MRID 47089401.

TABLE 2b. Observed Signs of Toxicity After Second Application (Day 7)		
Group	**Signs in the 4-hour observation period**	**Signs in the two days following treatment**
Control (B)	None reported.	Male #776 had tremors, slowness of movement, vomiting & unsteadiness on days 8 and/or 9. Male #801 vomited on day 8.
5X Test Material (A)	One male (#792) showed depression, rapid respiration and vomiting; however, this male had also shown these signs shortly before dosage.	Male #792 showed rapid respiration, slowness of movement and unsteadiness on day 8. This kitten received 24 mL of Lactated Ringers solution + 2 mg Ceftiofur + 0.5 mg Re-Covr subcutaneously. Another male (#775) showed slowness of movement, apprehensiveness, diarrhea and depression on days 8 and/or 9. On study day 9 #775 received 24 mL Lactated Ringers Solution, 2 mg Ceftiofur + 0.5 mg Re-Covr subcutaneously; also 2 mg Ceftiofur on study day 10. It is stated that symptoms for #775 may have been due to bacterial enteritis.

Data from information on p. 38 of MRID 47089401.

TABLE 2c. Observed Signs of Toxicity After Third Application (Day 14)		
Group	**Signs in the 4-hour observation period**	**Signs in the two days following treatment**
Control (B)	One male (#776) showed sneezing; one female (#800) showed pruritus.	One male (#776) showed depression, tremors and disorientation on days 15 and/or 16, and had dilated pupils on days 16 and 17.
5X Test Material (A)	Two females (#773, #794) showed pruritus.	None reported.

Data from information on p. 39 of MRID 47089401.

TABLE 2d. Observed Signs of Toxicity After Fourth Application (Day 21)		
Group	**Signs in the 4-hour observation period**	**Signs in the two days following treatment**
Control (B)	None reported.	One female (#788) showed tremors on days 22 and 23; had dilated pupils, unsteadiness and was apprehensive on day 23.
5X Test Material (A)	None reported.	None reported.

Data from information on p. 39 of MRID 47089401.

D. NEUROLOGICAL OBSERVATIONS

Some of the effects (tremors, disorientation, dilated pupils) observed in Group B kittens were consistent with neurotoxicity

E. BODY WEIGHT AND WEIGHT GAIN

All surviving kittens (in both the control and test material groups) had good weight gains from day -1 to 13, from day 13 to 28, and again from day 28 to 38. Two of the kittens, male #782 (assigned to the test material group) and male #791 (assigned to the control or solvent group) had slight weight losses pre-exposure. Male #782 went from 1.23 lbs on study day -7 to 1.14 lbs on study day -1, while #791 went from 1.06 lbs on study day -14 to 1.05 lbs on study day -7.

F. FOOD CONSUMPTION

No information is provided as to the exact (or even approximate) amount of food that was offered to kittens on a daily basis. As agreed with EPA, the amount of food consumed was determined visually, with 1 representing $\geq$ 75% offered food consumed, 2 being 25-75% consumption, and 3 representing <25% consumption. Most of the food consumption values were "1." The only values of "3" in Group A (test material treated kittens) after Day 0 were with male #792 on days 7 and 8 (coinciding with depression, rapid respiration and vomiting; signs observed prior to dosing on Day 7), and in male #775 on Day 9. For Group B there were two sporadic occurrences of "3" (male #776 on Day 9, correlating with other effects noted on Days 8-9; and female #793 on Day 24).

G. HEMATOLOGY

There were no physiologically significant changes or variations in hematology values.

H. CLINICAL CHEMISTRY

In the two male solvent control kittens (#783 and #791) which died on Day 1, there were a number of physiologically significant blood chemistry changes, including increases in aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline

phosphatase (ALP), and decreases in potassium (K), phosphorus (P) and calcium (Ca).

TABLE 3. Blood Chemistry Changes in Kittens Dying on Day 1

Kitten + Parameter	Day -7 Value	Day -1 Value	Day 1 Value
#783:			
Neutrophils	5.40 x 10^3/µL	11.71 x 10^3/µL	**34.67** x 10^3/µL
Potassium (K)	5.10 mmol/L	5.60 mmol/L	**3.30** mmol/L
Phosphorus (P)	8.30 mg/dL	7.80 mg/dL	**4.40** mg/dL
Calcium (Ca)	11.00 mg/dL	11.00 mg/dL	**5.90** mg/dL
ALP	213 u/L	231 u/L	**371** u/L
AST	22 u/L	22 u/L	**161** u/L
ALT	53 u/L	66 u/L	**396** u/L
#791:			
Neutrophils	4.62 x 10^3/µL	7.00 x 10^3/µL	**40.43** x 10^3/µL
Potassium (K)	4.50 mmol/L	6.20 mmol/L	**3.60** mmol/L
Phosphorus (P)	7.10 mg/dL	8.40 mg/dL	**4.90** mg/dL
Calcium (Ca)	10.60 mg/dL	10.80 mg/dL	**6.90** mg/dL
ALP	91 u/L	86 u/L	**437** u/L
AST	27 u/L	34 u/L	**251** u/L
ALT	29 u/L	25 u/L	**51** u/L

Data from information on p. 71 & 78 of MRID 47089401.

Female solvent control kitten (#788) with tremors on days 22 and 23 and dilated pupils, unsteadiness and apprehensiveness on day 23 had noticeably reduced potassium, phosphorus and calcium levels on Day 22. In addition, female solvent control kitten #797 (no signs of toxicity) had slightly reduced potassium and somewhat elevated AST and ALT on Day 22. Values were within normal ranges for both kittens on Day 38.

TABLE 4. Blood Chemistry Values in #788 & #797

Kitten + Parameter	Day -7	Day -1	Day 1	Day 22	Day 38
#788:					
Neutrophils	7.35 x 10^3/µL	7.69 x 10^3/µL	12.23x10^3/µL	**16.83**x10^3/µL	7.22 x 10^3/µL
Potassium (K)	7.10 mmol/L	6.50 mmol/L	6.30 mmol/L	**3.90** mmol/L	4.90 mmol/L
Phosphorus (P)	9.30 mg/dL	8.60 mg/dL	9.00 mg/dL	**6.70** mg/dL	7.20 mg/dL
Calcium (Ca)	11.00 mg/dL	10.80 mg/dL	11.20 mg/dL	**8.00** mg/dL	10.60 mg/dL
ALP	53 u/L	113 u/L	119 u/L	134 u/L	102 u/L
AST	52 u/L	31 u/L	33 u/L	38 u/L	17 u/L
ALT	76 u/L	57 u/L	62 u/L	76 u/L	60 u/L
#797:					
Neutrophils	6.42 x 10^3/µL	12.67x10^3/µL	14.19x10^3/µL	10.11x10^3/µL	27.26x10^3/µL
Potassium (K)	6.80 mmol/L	5.40 mmol/L	4.90 mmol/L	**3.80** mmol/L	4.90 mmol/L
Phosphorus (P)	9.10 mg/dL	8.80 mg/dL	8.00 mg/dL	8.10 mg/dL	7.90 mg/dL
Calcium (Ca)	10.20 mg/dL	10.80 mg/dL	10.80 mg/dL	10.60 mg/dL	10.30 mg/dL
ALP	137 u/L	167 u/L	183 u/L	151 u/L	159 u/L
AST	33 u/L	19 u/L	24 u/L	**48** u/L	24 u/L
ALT	91 u/L	46 u/L	50 u/L	**108** u/L	57 u/L

Data from information on p. 76 & 83 of MRID 47089401.

I. NECROPSY FINDINGS

At gross necropsy both of the two male kittens of the control group which died on Day 1 had distended urinary bladders. On microscopic examination, both showed necrosis in the external granular layer of the cerebellum and lymphoid tissues. Other findings were incidental and/or considered to be secondary to the moribund condition of the kittens.

IV. DISCUSSION

In a companion animal safety study (MRID 47089401), there were two groups, each containing 7 male and 7 female kittens (from 7 weeks 5 days to 8 weeks old; day -1 bodyweights: males: 1.14-2.09 lbs; females: 1.42-2.10 lbs; source: Harlan Sprague Dawley, Inc., Madison, WI). Kittens in Group A were treated with the formulation containing actives (Imidacloprid: 9.1%; Pyriproxyfen: 0.9%) at 5X the label-specified use application rate of 0.4 mL (5 x 0.4 mL = 2.0 mL) while kittens in Group B were treated with 2.0 mL of vehicle (equal to ~5.6X the amount of solvents and non-active ingredients from a single use application of the proposed product). The dose was applied topically on the backside of the head and the neck of each kitten to avoid runoff. Kittens were treated on Days 0, 7, 14 and 21; the proposed label indicates once-a-month treatment, so that each of the kittens in Group A received a cumulative total of 20X of the proposed monthly dosage of the formulation while kittens in Group B received a cumulative total of ~22.4X of the proposed monthly dosage of solvents and non-active ingredients. At the end of the study the heaviest kitten was 3.93 lbs, so none reached a weight >9 lbs (4.1 kg) which would have resulted in an increase in the 1X dose from 0.4 to 0.8 mL.

On the days of dosing (Days 0, 7, 14 and 21) each kitten was observed five times, once prior to dosage and then at hourly intervals for four hours after application. Otherwise, clinical observations were made twice a day. Individual daily food consumption was determined visually, using a scoring system ($\geq$75% consumption = 1, 25-75% consumption = 2, $\leq$25% consumption = 3). The kittens were weighed at six times before and during the study (Days -14, -7, -1, 13, 28 and 38). Blood samples were taken on Days -7, -1, 1, 22, and 38. Blood samples from four kittens were also collected on study day 2, from 3 kittens on study days 22-23 (insufficient initial samples and machine malfunction) and blood was collected a second time from one kitten on day 38 (due to clotting). Prothrombin time and activated partial thromboplastic time measurements were not done because of the comparatively large amount of blood required for these tests and the age of the kittens; this protocol deviation had been previously discussed with and accepted by the Agency.

Signs of toxicity in Group A kittens following the Day 0 treatment were salivation (2 females) and salivation accompanied by sneezing (one male). One of the two affected females had nasal discharge on Day 1. Following treatment on Day 7 one male (#792) showed depression, rapid respiration and vomiting; however, this male had also shown these signs shortly before dosage. Male #792 subsequently showed rapid respiration, slowness of movement and unsteadiness on Day 8, and received 24 mL Lactated Ringers Solution (LRS) + 2 mg Ceftiofur + 0.5 mg Re-Covr. Following the Day 7 treatment another male (#775) was slow to move and depressed, and also had diarrhea on Days 8 and/or 9. On Day 9 #775 was treated with 24 mL LRS + 2 mg Ceftiofur + 0.5 mg Re-Covr, also 2 mg Ceftiofur on Day 10. It is stated that symptoms in #775 were not due to test material and may have been from bacterial enteritis. During the observation period following the Day 14 treatment 2 females showed pruritis.

Two male kittens (same sire, but from different mothers) in Group B had adverse reactions to the solvent control (vehicle) and died on Day 1. Signs observed prior to death in one of the kittens were sneezing, salivation, dilated pupils, rapid breathing, generalized tremors

and slow movement. Signs in the other kitten were dilated pupils, rapid breathing and generalized tremors. In the blood samples taken on day 1 both of these male kittens showed a number of physiologically significant hematology and blood chemistry changes, including increases in neutrophils, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP), and decreases in potassium (K), phosphorus (P) and calcium (Ca). At gross necropsy both had distended urinary bladders. On microscopic examination, both showed necrosis in the external granular layer of the cerebellum and lymphoid tissues. Other necropsy findings were incidental and/or considered to be secondary to the moribund condition of the kittens.

There were no signs of toxicity in other Group B kittens following the Day 0 treatment. Following the Day 7 treatment one Group B male had a number of signs which included tremors, slowness of movement, vomiting and dilated pupils on Days 8-9. The same male also had sneezing following the application on Day 14, followed by depression, tremors, disorientation and dilated pupils on Days 16-18. This male (#776) had a different sire from that of the two kittens which died. One female showed pruritis in the observation period following the Day 14 treatment.

One female (#788) in Group B had tremors on days 22 and 23 and dilated pupils, unsteadiness and apprehensiveness on day 23, and also had noticeably reduced potassium, phosphorus and calcium levels on Day 22. In addition, female solvent control kitten #797 (not reported as having any signs of toxicity) had slightly reduced potassium and somewhat elevated ASP and ALT on Day 22. Values were within normal ranges for both of these kittens on Day 38. One possible explanation for the greater severity of symptoms seen in the Group B (solvent control) kittens was that the toxicity was a result of oral ingestion of the solvent(s), but that a bad taste or some other unpleasant sensation associated with the active ingredients resulted in considerably less oral ingestion of the test material in the Group A kittens.

This study is classified as **Supplementary** as a companion animal safety study (OPPTS 870.7200), as it does not demonstrate a 5X margin of safety associated with exposure to the solvents/inerts and the proposed use of the formulation (with actives) in 8 week old kittens.

ACUTE TOX ONE-LINERS

1. **DP BARCODE:** D338710
2. **PC CODES:** 129099 (Imidacloprid: 9.1%); 129032 (Pyriproxyfen: 0.9%)
3. **CURRENT DATE:** 5 September 2007
4. **TEST MATERIALS:** 9.1% Imidacloprid with 0.9% Pyriproxyfen Spot-On Formulation, a liquid with a specific gravity of 1.097 g/mL, Lot No. 99-901-66; also tested was the test material without the actives, a liquid with a specific gravity of 1.0674 g/mL, Lot No. 99-901-68.

Study/Species/Lab Study # /Date	MRID	Results	Tox. Cat.	Core Grade
Companion animal safety / 8-week old kitten/ Intervet Inc., DeSoto, KS /Project ID 75120 (150.851) / 8-JUN-2001	47089401	2 groups, each containing 7M & 7F 8-week old domestic short-hair cats were used. Kittens in Group A were treated with 5X application levels of the formulation containing the actives on Days 0, 7, 14, 21. Kittens in Group B were treated with ~5.6X application levels of the formulation without the actives. Two male kittens in Group B had adverse reactions (dilated pupils, generalized tremors, rapid breathing) and were euthanized on Day 1. At gross necropsy both had distended urinary bladders. On microscopic examination both showed necrosis of the external granular layer of the cerebellum. A number of Day 1 hematology & clinical chemistry changes were seen in the two dead kittens (increased neutrophil counts, decreased calcium, phosphorus, increased ALP, AST & ALT). Some other Group B kittens (but no group A kittens) had tremors following Day 7 and 21 treatment, with suggestive changes in clinical chemistry parameters. Study does not demonstrate 5X safety factor for exposure to solvents/inerts of this formulation.	N/A	S

Core Grade Key: A =Acceptable, S = Supplementary, U = Unacceptable, W = Waived, I = Invalid

EPA Primary Reviewer: Byron T. Backus, Ph.D.
Technical Review Branch, Registration Division (7505P)
EPA Secondary Reviewer: Masih Hashim, D.V.M., Ph.D.
Technical Review Branch, Registration Division (7505P)

Signature: Byron T. Backus
Date: 9/06/2007
Signature: Masih Hashim 9/6/07
Date:

DATA EVALUATION RECORD

STUDY TYPE: Companion Animal Safety - Kittens (OPPTS 870.7200)

PC CODES: [129099 (Imidacloprid); 129032 (Pyriproxyfen)] – Not tested in this study

DP BARCODE: D338710

DECISION NO.: 215319

RISK MANAGER: (EPA): 01

TEST MATERIAL AND PRODUCT: The proposed product contains Imidacloprid (9.1%) and Pyriproxyfen (0.46%), as well as one or more solvents. What was tested in this study was the formulation without active ingredients, but with added water (4.6% of the resulting formulation).

CITATION: Abraham, A. S. (2001). Evaluation of the General Safety of 9.1% Imidacloprid with 0.45% Pyriproxyfen Spot-on with 5.0% Water Blank Formulation in the Target Species, 8-Week Old Kittens. Performing Laboratory: Intervet Inc. (formerly a facility owned and operated by Bayer Corporation Agriculture Division Animal Health), DeSoto Research Facility, DeSoto, Kansas 66018. Laboratory Project ID 75190 (150.828). Study Dated May 10, 2001. MRID 47089403. 165 p. + a 2 p. confidential appendix.

SPONSOR: Bayer Corporation Agriculture Division

SUBMITTER: Bayer HealthCare LLC, Animal Health Division, P.O. Box 390, Shawnee, KS 66201

EXECUTIVE SUMMARY: In a companion animal safety study (MRID 47089403), there were two groups, each containing 8 male and 8 female kittens (from 7 weeks 6 days to 8 weeks old at first dosing; day -1 bodyweights: males: 1.55-1.99 lbs; females: 1.47-1.96 lbs; source: Liberty Research Inc., Waverly, NY). Kittens in Group A were treated with the proposed formulation without the actives (but with 4.6% added water) at 5X the label indicated exposure rate for solvents (5 x [0.4 – 0.04] mL = 1.8 mL; this does not correct for the added water) while kittens in Group B received no treatment and served as controls.

The dose was applied topically on the backside of the head and the neck of each kitten to avoid runoff. Kittens were treated on Days 0, 7, 14 and 21; the proposed label indicates once-a-month treatment, so that each of the kittens in Group A received a cumulative total of 20X of the proposed monthly dosage of the formulation solvents. Kittens in Group B received no exposure to any test material. On Day 13 the heaviest kitten in Group A weighed 2.51 lbs, so none had reached a weight >9 lbs (4.1 kg) which would have resulted in an increase in the 1X dose from 0.36 to 0.72 mL (Day 13 weights were used to set the dosages for Days 14 and 21).

On the days of dosing (Days 0, 7, 14 and 21) each kitten was observed five times, once prior to dosage and then at hourly intervals for four hours after application. Otherwise, clinical observations were made twice a day. Individual daily food consumption was determined visually, using a scoring system (≥75% consumption = 1, 25-75% consumption = 2, ≤25% consumption = 3). The kittens

were weighed six times before and during the study (Days -14, -7, -1, 13, 28 and 35). Blood samples were taken on Days -7, -1, 1, 22, and 35. Blood samples were also collected from one or more kittens on study days -5, 23 and 36, because either insufficient blood had been collected the previous day or there had been clotting in the previous day's sample. Prothrombin time and activated partial thromboplastic time measurements were not done because of the comparatively large amount of blood required for these tests and the age of the kittens; this protocol deviation had been previously discussed with and accepted by the Agency.

Two Group A kittens (male #811, female #816, from the same litter) had adverse reactions to the test material (formulation solvents) and were euthanized on Day 1. Signs observed following dosage in male #811 were salivation; no signs (other than rough coat, observed in all Group A kittens following treatment) were observed in the 4 hours following dosage for female #816. Signs of toxicity on Day 1 in male #811 were tremors, incoordination, unsteadiness, apprehensiveness, depression and dilated pupils. Signs of toxicity on Day 1 in female #816 were rapid respiration, apprehensiveness, incoordination, tremors, depression, seizure and dilated pupils. In the blood samples taken on day 1 both of these kittens showed a number of physiologically significant blood chemistry changes, including increases in aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP), and decreases in phosphorus (P) and calcium (Ca). Both also showed an increase (72-82%) in neutrophils from the Day -1 measurement. Kitten #811 showed an approximately 30% drop in potassium (K) from the Day -1 measurement. At gross necropsy both had distended urinary bladders, mottled (red and tan) livers, and pale kidneys. On microscopic examination, both showed necrosis in the external granular layer of the cerebellum.

There were no observed signs of treatment-related toxicity in other Group A kittens following the Day 0 treatment, including female #814, which was a littermate of #811 and #816. However, following the Day 7 treatment female #814 was unsteady and had tremors on Day 8, had tremors, circling and slowness on Day 9, and showed unsteadiness and sneezing on Day 10, but recovered on Day 11. None of the Group A kittens (including #814) showed signs of toxicity following treatment on Days 14 and 21. Kitten #814 had some clinical chemistry values (including elevated values for ALP, AST and ALT) on Day 1 similar – but not as pronounced – to those seen in #811 and #816.

Group A kittens all showed a rough hair coat (localized effect?) following applications on Days 0, 7, 14 and 21. Both Group A and Group B kittens showed sporadic episodes of loose (soft) stools, and Group A male #828 vomited prior to dosing on Day 14.

This study is classified as **Supplementary** as a companion animal safety study (OPPTS 870.7200), because it did not include testing of the proposed formulation with actives and because it does not indicate an adequate (5X) margin of exposure exists between the application rate exposure level to the solvent(s) of the proposed product and that which can result in the death of some kittens. The results of this study are consistent with the findings of the study in MRID 47089401, in which two kittens dosed at 5.6X with the solvent control formulation died following treatment on Day 0.

COMPLIANCE: Signed and dated Quality Assurance (p. 4), [No] Data Confidentiality (p. 2) and Good Laboratory Practice Compliance (p. 3) statements are present.

I. MATERIAL

MATERIALS

1. Test material: The test material without the 2 active ingredients (9.1% Imidacloprid and 0.46% Pyriproxyfen), but with 4.6% water added..
   Description: A liquid, specific gravity not reported.
   Lot No.: 99-901-101.
   Storage: Stored in amber glass bottles at room temperature.
   Placebo: None (controls were not exposed to any test substance)
   Description: N/A
   Lot No.: N/A
   Storage: N/A

2. Administration: Topically applied to the backside of the head and the neck of each kitten to avoid run off.

3. Test animals
   Species: Cat
   Breed: Domestic Short hair
   Ages and weights at study initiation (Day 0 for ages, Day -1 for weights): 7 weeks 6 days to 8 weeks; males: 1.55 to 1.99 lbs; females: 1.47 to 1.96 lbs.
   Source: Liberty Research Inc., Waverly, NY
   Vaccinations and other medications: The kittens had been vaccinated with a four way feline vaccine, Fel-O-Vax IV (Feline Rhinotracheitis, Calici, Panleukopenia, Chlamydia Psittaci, Killed Virus and Chlamydia) on Study Days -14 and -7. All the kittens had been treated for coccidiosis once daily orally with Albon Suspension (Sulfadimethoxine) from study day -14 through study day -7. On Study Day -14, each kitten received approximately 25 mg of Sulfadimethoxine per pound of body weight. From Study Day -13 to -7 each kitten received approximately 12.5 mg of Sulfadimethoxine per pound of body weight/day.
   Housing: individual in cages with approximately 7.5 ft^2 of floor space per cage.
   Diet: Harlan Teklad® (commercial dry cat feed) and a canned kitten food (Feline Growth) from Hill's Pet Nutrition, Kansas City, MO. (fed once daily, however, no information is provided as to the amount that was offered). The kittens were fed with canned food until Day 22.
   Water: Tap water, *ad libitum*
   Environmental conditions:
      Temperature: (not stated)
      Humidity: (not stated)
      Air changes: (not stated)
      Photoperiod: 9-14 hours of lighting/day
   Acclimation period: 14 days

II. STUDY DESIGN

A. IN LIFE DATES

From the report (p. 12 of MRID 47089403) Day 0 was December 13, 1999. The experimental phase of the study was completed on February 28, 2000 (histopathology slides read).

B. ANIMAL ASSIGNMENT/ DOSAGE AND ADMINISTRATION

From p. 14 of MRID 47089403: "Thirty-two animals [out of a total of 44] were randomly allocated to two groups. Animals were blocked by sex and ranked by ascending order of study day -1 body weight and assigned a random number. From the first block (female), the animal with the larger of the first two random numbers was assigned to Group A (test substance), and the smaller to Group B (negative controls) and so forth until all the animals in the same sex were assigned. This procedure was repeated for the males..."

From p. 19 of MRID 47089403: "Eight males and eight females in group A were dosed with 5 times the monthly use rate volume (1.8 mL for kittens up to 9 lbs) of test substance. This resulted in a 20X (5X per week for four consecutive weeks) the monthly use volume of vehicle applied in a month's time... Eight males and eight females in Group B received no treatment and served as negative controls."

From p. 19 of MRID 47089403: "The dose was administered topically on the backside of the head and the neck of each kitten [in Group A] to avoid run off of test substance... The kittens [in Group A] were dosed four times, on study days 0, 7, 14, and 21."

TABLE 1. Study design

Group & Weight Range (lb)			Mean Kitten Weight			
		Number of kittens	Mean Kitten Wt ± S.D. (lb) on Day -1 (before 1st application)	Mean Kitten Wt ± S.D. (lb) on Day 13 (before 3rd application)	Mean Kitten Wt ± S.D. (lb) on Study Day 28	Mean Kitten Wt ± S.D. (lb) on Study Day 37
(A): 5X solvent	males ≤ 9 lb	8*	1.75 ± 0.13	2.32 ± 0.11	2.96 ± 0.14	3.28 ± 0.16
	females ≤ 9 lb	8*	1.70 ± 0.17	2.22 ± 0.22	2.79 ± 0.20	3.05 ± 0.19
	combined ≤ 9 lb	16**	1.72 ± 0.15	2.27 ± 0.18	2.88 ± 0.18	3.17 ± 0.21
(B): no treatment	males ≤ 9 lb	8	1.74 ± 0.13	2.33 ± 0.08	2.99 ± 0.08	3.34 ± 0.08
	females ≤ 9 lb	8	1.71 ± 0.17	2.21 ± 0.18	2.80 ± 0.21	3.11 ± 0.25
	combined ≤ 9 lb	16	1.72 ± 0.15	2.27 ± 0.15	2.90 ± 0.18	3.22 ± 0.21

Data calculated from information on p. 32 and 34 of MRID 47089403.
*7 kittens on Days 13, 28 and 38, as two kittens had been euthanized on Day 1.
**14 kittens on Days 13, 28 and 38, as two kittens had been euthanized on Day 1.

C. DOSE SELECTION RATIONALE

From p. 11 of MRID 47089403: "The study was conducted to evaluate the safety of 9.1% Imidacloprid with 0.46% Pyriproxyfen (w/w) spot-on with 4.6% water blank formulation on kittens applied at 5 times the use volume at weekly intervals for a total of 4 weeks. This study was designed as a limit test and a full study with three dose levels at 1X, 3X and 5X was not conducted. Only blank formulation without active ingredient group was included in the study..."

D. EXPERIMENTAL DESIGN

There were two groups, each initially containing 8 male and 8 female kittens (from 7 weeks 6 days to 8 weeks old; day -1 bodyweights: males: 1.55-1.99 lbs; females: 1.47-1.96 lbs). Kittens in Group A were treated with the proposed product without the actives (and with 4.6% water) at 5X the label-specified use exposure rate for solvents (5 x [0.4-0.04 mL] = 1.8 mL) while kittens in Group B were not treated with anything. The dose was applied topically on the backside of the head and the neck of each kitten in Group A to avoid runoff. The dose was administered by parting the hair and using a syringe without a needle. Kittens were treated on Days 0, 7, 14 and 21; since the proposed label indicates once-a-month treatment, each of the kittens in Group A received a cumulative total of 20X of the proposed monthly dosage of the formulation solvents. Group B kittens served as negative controls.

At the last weighing (Day 35) the heaviest kitten weighed 3.54 lbs, so none had reached a weight >9 lbs (4.1 kg) which would have resulted in an increase in the 5X dose from 2.0 to 4.0 mL.

On the days of dosing (Days 0, 7, 14 and 21) each kitten was observed five times, once prior to dosage and then at hourly intervals for four hours after application. Otherwise, clinical observations were made twice (once in the a.m., once in the p.m.) a day. Individual daily food consumption was determined visually, using a scoring system ($\geq$75% consumption = 1, 25-75% consumption = 2, $\leq$25% consumption = 3). The kittens were weighed at six times before and during the study (Days -14, -7, -1, 13, 28 and 35). Blood samples were taken on Days -7, -1, 1, 22, and 35. It was necessary to obtain additional blood samples from individual kittens on study days -5, 23 and 36 due to insufficient quantities initially obtained or because of blood sample clotting. Prothrombin time and activated partial thromboplastic time measurements were not done because of the comparatively large amount of blood required for these tests and the age of the kittens; this protocol deviation had been previously discussed with and accepted by the Agency.

From p. 21 of MRID 47089403: "Physical examinations were performed on the study animals on study days -14, -1, and 35."

E. CLINICAL PATHOLOGY PARAMETERS

Blood samples were collected from each kitten on study Days -7, -1, 1, 22 and 35. There is no indication within the report that kittens were fasted prior to collection of blood. The CHECKED (X) parameters were examined:

a. Hematology

X		X	
X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Absolute and percent basophil count
		X	Absolute and percent eosinophil count
X	Leukocyte count (WBC)*	X	Absolute and percent lymphocyte count
X	Erythrocyte count (RBC)*	X	Absolute and percent monocyte count
		X	Absolute and percent neutrophil count
X	Platelet count (PLTS)	X	Mean corpuscular HGB (MCH)*
	Blood clotting measurements	X	Mean corpusc. HGB conc.(MCHC)*
	(Thromboplastin time)	X	Mean corpusc. volume (MCV)*
	(Clotting time)		
	(Prothrombin time [PT])*		
	(Activated partial thromboplastin time [APTT])*		

*Recommended in OPPTS 870.7200 Guidelines. The Prothrombin time and Activated partial thromoplastin time were not done because of the comparatively large volume of blood required for these tests and the age of the kittens. This deviation had been accepted by the Agency prior to initiation of this study.

b. Clinical chemistry

X	ELECTROLYTES	X	OTHER
X	Calcium*	X	Albumin (Alb)*
X	Chloride*	X	Creatinine (Crea)*
	Magnesium	X	Blood urea nitrogen (BUN)*
X	Phosphorus*		Total Cholesterol
X	Potassium*	X	Globulin (Glob)*
X	Sodium*	X	Glucose (Gluc)*
		X	Total bilirubin (T Bil)*
	ENZYMES	X	Direct bilirubin (D Bil)*
			Total protein (TP)*
X	Alkaline phosphatase(ALP or ALK)*		Triglycerides
	Cholinesterase(ChE)		Serum protein electrophoresis
	Creatine phosphokinase	X	Albumin/Globulin (A/G) ratio
	Lactic acid dehydrogenase(LDH)		Lipase
X	Serum alanine aminotransferase (ALT or SGPT)*		
X	Serum aspartate aminotransferase(AST or SGOT)*	X	BUN/Creatinine ratio
		X	Ca/Phos Ratio
	Gamma glutamyl transpeptidase(GGT)	X	Na/K Ratio
	Amylase		

*Recommended in OPPTS 870.7200 Guidelines.

F. CONCOMITANT MEDICATIONS/THERAPIES

From p. 19 of MRID 47089403: "All the kittens were treated for coccidiosis once daily orally with Albon Suspension (Sulfadimethoxine) from study day -14 through study day -7. On study day -14, the animals received approximately 25 mg of Sulfadimethoxine per pound of body weight. Subsequent days (study day -13 to -7) the animals received approximately 12.5 mg of Sulfadimethoxine per pound of body weight."

G. STATISTICS

Although means and standard deviations were calculated for some parameters (such as body weight), statistical tests were primarily applied to chemistry and hematology pathology parameters. From p. 143 of MRID 47089403: "This study is intended to confirm the general safety of the vehicle and approximately the same number of adverse effects is expected between the two groups. Adverse effects will be summarized in tables. Certain types of adverse effects may be grouped together, depending on the clinical presentation, such as all effects, all transient effects or all blood chemistry effects. If the number or pattern of effects elicit clinical interest, incidence rates will also be compared between groups." For the clinical pathology parameters, it is stated (p. 21 of MRID 47089403): "For each animal, a baseline value was calculated for each clinical pathology test, by averaging the two pretreatment measurements (study days -7 and -1). Each clinical pathology test was then analyzed with a multivariate repeated measures ANOVA (baseline, study days 1, 22, and 35) including terms for Group, Sex, Animal (random), Day, and Group*Day as the predictors..."

H. DISPOSITION OF ANIMALS

From p. 14 of MRID 47089403: "Twelve animals not included in the experimental phase of the study were euthanized on December 13, 1999. Two [Group A] kittens were euthanized on December 14. [19]99 (Study Day 1). Twenty-nine animals in the experimental phase of the study were euthanized on January 21, 2000. One animal in the experimental phase of the study was given for adoption as pet." According to the OPPTS 870.7200 Guidelines: "Routine sacrifice or necropsy is not required for surviving animals."

I. COMPLIANCE

Signed and dated Quality Assurance [p. 4], [No] Data Confidentiality [p. 2], and Good Laboratory Practice (GLP) Compliance [p. 3] Statements were present.

III. RESULTS

A. EXPOSURE LEVELS

Refer to Table 1 of this DER. Kittens in the control group (Group B), all weighing $\leq$ 9.0 lb, were not dosed, while kittens in the test group (Group A), also all weighing $\leq$ 9.0 kg, were dosed with 1.8 mL of the formulation without actives (but with 4.6% added water) at each application. Applications were made on Days 0, 7, 14 and 21.

B. MORTALITY

Two Group A (male #811, female #816) kittens were euthanized during the afternoon of Day 1; all other kittens survived the 35-day observation period.

C. CLINICAL SIGNS

Group A: One male (#808) showed salivation at 3 hours post-dosing on Day 0. All kittens in Group A showed a rough hair coat at the 4 observations following dosage. One female (#823) and one male (#828) had loose stools on Day 1. Two kittens showed severe clinical signs on Day 1: male #811 had tremors, incoordination, unsteadiness, apprehension, depression and dilated pupils, while female #816 had rapid respiration, apprehension, incoordination, tremors, depression, seizures and dilated pupils. Both kittens showing severe clinical signs were euthanized during the afternoon of Day 1. Female #814 had loose stools on Day 4. Female #814 was unsteady and had tremors on Day 8, tremors, circling and slowness on Day 9, and unsteadiness and sneezing on Day 10. Group A kittens had rough hair coats following treatments on Days 7, 14 and 21.

Group B: Four kittens each had loose stools on three occasions during the period from study day 1 to 4. One kitten had loose stools on Day 14.

TABLE 2a. Observed Signs of Toxicity After First Application (Day 0)

Group	Signs in the 4-hour observation period	Signs in the 1-3 days following treatment
5X Vehicle (A)	Diarrhea in one female (#814) Sneezing in one male (#808) Salivation in one male (#811) Loose (soft) stool in one male (#828) All treated kittens with rough hair coat following application.	Female #816 on study day 1: rapid respiration, apprehensiveness, incoordination, tremors, depression, seizure, dilated pupils – euthanized 3:20 PM. Male #811 on study day 1: tremors, incoordination, unsteadiness, apprehensiveness, , depression, dilated pupils – euthanized 3:19 PM Female #823 & Male #828: Loose stools
No Treatment (B)	Loose (soft) stools in females #815, #847. Diarrhea in male #821.	Four kittens (females #813, #815, and #847; male # 821) with loose (soft) stools on days 1 and/or 2 and/or 3 and/or 4.

Data from information on p. 37 of MRID 47089403.

TABLE 2b. Observed Signs of Toxicity After Second Application (Day 7)		
Group	**Signs in the 4-hour observation period**	**Signs in the 1-3 days following treatment**
5X Vehicle (A)	All treated kittens with rough hair coat following application.	Female #814: unsteadiness & tremors on day 8; tremors, circling, slowness on day 9; unsteadiness & sneezing on day 10
No Treatment (B)	None	None.

Data from information on p. 38 of MRID 47089403.

TABLE 2c. Observed Signs of Toxicity After Third Application (Day 14)		
Group	**Signs in the 4-hour observation period**	**Signs in the 1-3 days following treatment**
5X Vehicle (A)	All treated kittens with rough hair coat following application.	None.
No Treatment (B)	Male #821: loose (soft) stools on day 14.	None.

Data from information on p. 38 of MRID 47089403.

TABLE 2d. Observed Signs of Toxicity After Fourth Application (Day 21)		
Group	**Signs in the 4-hour observation period**	**Signs in the 1-3 days following treatment**
5X Vehicle (A)	All treated kittens with rough hair coat following application.	None.
No Treatment (B)	None.	None.

Data from information on p. 38 of MRID 47089403.

D. NEUROLOGICAL OBSERVATIONS

Some of the effects (tremors, disorientation, dilated pupils) observed in Group A kittens were consistent with neurotoxicity

E. BODY WEIGHT AND WEIGHT GAIN

All surviving kittens (in both groups) had good weight gains from day -1 to 13, from day 13 to 28, and again from day 28 to 35.

F. FOOD CONSUMPTION

No information is provided as to the exact (or even approximate) amount of food that was offered to kittens on a daily basis. As agreed with EPA, the amount of food consumed was determined visually, with 1 representing $\geq$ 75% offered food consumed, 2 being 25-75% consumption, and 3 representing <25% consumption. Most of the food consumption values were "1." The only value of "3" in Group A (vehicle treated kittens) after Day 0 was with female #816 on day 1 (the day this kitten was euthanized). A number of other Group A kittens (#814, #846 and #811) had values of "2" on day 1, but so did a number of group B kittens (#835, #818 and #821). Group A female #814 had a value of "2" on days 9 and 15, group A male #833 had a value of "2" on day 16, and group A female #846 had a value of "2" on day 23; otherwise, all group A kittens had values of "1" for food consumption in the period from day 2 to 35.

G. HEMATOLOGY

Day 1 neutrophil counts were noticeably elevated from their Day -1 values in the two euthanized kittens, #811 and #816 (kitten #811: 6.44 x 10^3/µL on Day -1; 11.71 x 10^3/µL on Day 1; kitten #816: 13.4 x 10^3/µL on Day -1; 23.03 x 10^3/µL on Day 1).

H. CLINICAL CHEMISTRY

In the two Group A kittens (#811 and #816) which were euthanized on Day 1, there were a number of physiologically significant blood chemistry changes, including increases in glucose, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline

phosphatase (ALP), and decreases in potassium (K), phosphorus (P) and calcium (Ca).

TABLE 3. Selected Hematology and Blood Chemistry Values for Kittens Euthanized on Day 1

Kitten + Parameter	Day -7 Value	Day -1 Value	Day 1 Value
#811:			
Neutrophils	6.77 x 10^3/µL	6.44 x 10^3/µL	**11.71 x 10^3**/µL
Glucose	110 mg/dL	125 mg/dL	**134** mg/dL
Potassium (K)	5.7 mmol/L	4.8 mmol/L	**4.5** mmol/L
Phosphorus (P)	9.8 mg/dL	9.3 mg/dL	**6.2** mg/dL
Calcium (Ca)	11.2 mg/dL	11.9 mg/dL	**5.1** mg/dL
ALP	94 u/L	100 u/L	**156** u/L
AST	19 u/L	22 u/L	**243** u/L
ALT	35 u/L	39 u/L	**360** u/L
#816:			
Neutrophils	9.81 x 10^3/µL	13.4 x 10^3/µL	**23.03 x 10^3**/µL
Glucose	91 mg/dL	111 mg/dL	**188** mg/dL
Potassium (K)	7.2 mmol/L	6.9 mmol/L	**4.8** mmol/L
Phosphorus (P)	10.9 mg/dL	10.8 mg/dL	**4.9** mg/dL
Calcium (Ca)	11.7 mg/dL	12.5 mg/dL	**5.3** mg/dL
ALP	125 u/L	105 u/L	**197** u/L
AST	24 u/L	26 u/L	**633** u/L
ALT	33 u/L	35 u/L	**1333** u/L

Data from information on p. 71 & 75 of MRID 47089403.

Group A female #814, which had symptoms (including tremors) on days 8-10, had slight decreases in blood phosphorus and calcium, and noticeable increases in ALP, AST and ALT on Day 1, but these values were normal for this kitten on Day 22. Kitten #814 is identified (p. 31 of MRID 47089403) as being a littermate of #811 and #816. None of the remaining kittens from this litter was in Group A (#813 and #815 were in Group B; #812 was received but not included in this study).

TABLE 4. Selected Hematology and Blood Chemistry Values for #814

Kitten + Parameter	Day -7	Day -1	Day 1	Day 22	Day 35
#814:					
Neutrophils	7.09 x10^3/µL	11.93x10^3/µL	**13.37x10^3**/µL	9.41 x 10^3/µL	8.88 x 10^3/µL
Glucose	80 mg/dL	91 mg/dL	89 mg/dL	96 mg/dL	87 mg/dL
Potassium (K)	6.3 mmol/L	6.6 mmol/L	**5.8** mmol/L	6.7 mmol/L	7.6 mmol/L
Phosphorus (P)	9.4 mg/dL	11 mg/dL	**8** mg/dL	10 mg/dL	9.9 mg/dL
Calcium (Ca)	12.2 mg/dL	12.9 mg/dL	**10.4** mg/dL	11.8 mg/dL	11.6 mg/dL
ALP	41 u/L	27 u/L	**95** u/L	25 u/L	22 u/L
AST	41 u/L	27 u/L	**95** u/L	25 u/L	22 u/L
ALT	50 u/L	57 u/L	**157** u/L	60 u/L	55 u/L

Data from information on p. 73 of MRID 47089403.

I. NECROPSY FINDINGS

At gross necropsy both of the kittens which were euthanized on Day 1 had distended urinary bladders. On microscopic examination, both showed necrosis in the external granular layer of the cerebellum. Other findings were incidental and/or considered to be secondary to the moribund condition of the kittens.

IV. DISCUSSION

In a companion animal safety study (MRID 47089403), there were two groups, each containing 8 male and 8 female kittens (from 7 weeks 6 days to 8 weeks old at first dosing; day -1 bodyweights: males: 1.55-1.99 lbs; females: 1.47-1.96 lbs; source: Liberty Research Inc., Waverly, NY). Kittens in Group A were treated with the proposed formulation without the actives (but with 4.6% added water) at 5X the label indicated exposure rate for solvents (5 x [0.4 – 0.04] mL = 1.8 mL; this does not correct for the added water) while kittens in Group B received no treatment and served as controls.

The dose was applied topically on the backside of the head and the neck of each kitten to avoid runoff. Kittens were treated on Days 0, 7, 14 and 21; the proposed label indicates once-a-month treatment, so that each of the kittens in Group A received a cumulative total of 20X of the proposed monthly dosage of the formulation solvents. Kittens in Group B received no exposure to any test material. On Day 13 the heaviest kitten in Group A weighed 2.51 lbs, so none had reached a weight >9 lbs (4.1 kg) which would have resulted in an increase in the 1X dose from 0.36 to 0.72 mL (Day 13 weights were used to set the dosages for Days 14 and 21).

On the days of dosing (Days 0, 7, 14 and 21) each kitten was observed five times, once prior to dosage and then at hourly intervals for four hours after application. Otherwise, clinical observations were made twice a day. Individual daily food consumption was determined visually, using a scoring system ($\geq$75% consumption = 1, 25-75% consumption = 2, $\leq$25% consumption = 3). The kittens were weighed six times before and during the study (Days -14, -7, -1, 13, 28 and 35). Blood samples were taken on Days -7, -1, 1, 22, and 35. Blood samples were also collected from one or more kittens on study days -5, 23 and 36, because either insufficient blood had been collected the previous day or there had been clotting in the previous day's sample. Prothrombin time and activated partial thromboplastic time measurements were not done because of the comparatively large amount of blood required for these tests and the age of the kittens; this protocol deviation had been previously discussed with and accepted by the Agency.

Two Group A kittens (male #811, female #816, from the same litter) had adverse reactions to the test material (formulation solvents) and were euthanized on Day 1. Signs observed following dosage in male #811 were salivation; no signs (other than rough coat, observed in all Group A kittens following treatment) were observed in the 4 hours following dosage for female #816. Signs of toxicity on Day 1 in male #811 were tremors, incoordination, unsteadiness, apprehensiveness, depression and dilated pupils. Signs of toxicity on Day 1 in female #816 were rapid respiration, apprehensiveness, incoordination, tremors, depression, seizure and dilated pupils. In the blood samples taken on day 1 both of these kittens showed a number of physiologically significant blood chemistry changes, including increases in aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP), and decreases in phosphorus (P) and calcium (Ca). Both also showed an increase (72-82%) in neutrophils from the Day -1 measurement. Kitten #811 showed an approximately 30% drop in potassium (K) from the Day -1 measurement. At gross necropsy both had distended urinary bladders, mottled (red and tan) livers, and pale

kidneys. On microscopic examination, both showed necrosis in the external granular layer of the cerebellum.

There were no observed signs of treatment-related toxicity in other Group A kittens following the Day 0 treatment, including female #814, which was a littermate of #811 and #816. However, following the Day 7 treatment female #814 was unsteady and had tremors on Day 8, had tremors, circling and slowness on Day 9, and showed unsteadiness and sneezing on Day 10, but recovered on Day 11. None of the Group A kittens (including #814) showed signs of toxicity following treatment on Days 14 and 21. Kitten #814 had some clinical chemistry values (including elevated values for ALP, AST and ALT) on Day 1 similar – but not as pronounced – to those seen in #811 and #816.

Group A kittens all showed a rough hair coat (localized effect?) following applications on Days 0, 7, 14 and 21. Both Group A and Group B kittens showed sporadic episodes of loose (soft) stools, and Group A male #828 vomited prior to dosing on Day 14.

This study is classified as **Supplementary** as a companion animal safety study (OPPTS 870.7200), because it did not include testing of the proposed formulation with actives and because it does not indicate an adequate (5X) margin of exposure exists between the application exposure level to the solvent(s) of the proposed product and that which can result in the death of some kittens. The results of this study are consistent with the findings of the study in MRID 47089401, in which two kittens dosed at 5.6X with the solvent control formulation died following treatment on Day 0.

ACUTE TOX ONE-LINERS

1. **DP BARCODE:** D338710
2. **PC CODES:** N/A (solvents only tested)
3. **CURRENT DATE:** 6 September 2007
4. **TEST MATERIAL:** The proposed product contains Imidacloprid (9.1%) and Pyriproxyfen (0.46%), as well as one or more solvents. What was tested in this study was the formulation without active ingredients, but with added water (4.6% of the resulting formulation).

Study/Species/Lab Study # /Date	MRID	Results	Tox. Cat.	Core Grade
Companion animal safety / 8-week old kitten / Intervet Inc., DeSoto, KS / Project ID 75190 (150.828) / 10-MAY-2001	47089403	2 groups, each containing 8M & 8F 8-week old domestic short-hair kittens were used. Kittens in Group A were treated with 5X application levels (=1.8mL) of the formulation without actives on days 0, 7, 14 & 21; kittens in Group B were not treated. Two Group A kittens (1M & 1F) had adverse reactions (tremors, incoordination, dilated pupils) and were euthanized on Day 1. Both kittens showed a number of significant Day 1 blood chemistry changes including increases in ALP, AST, ALT and decreases in serum phosphorus and calcium. On microscopic examination, both showed necrosis in the external granular layer of the cerebellum. Group A kitten #814 had similar – but not as pronounced symptoms – following the Day 7 treatment but recovered by Day 11. Results of study show a less than 5X margin of safety between the application rate exposure level to the solvents and that which can result in death.	N/A	S

Core Grade Key: A =Acceptable, S = Supplementary, U = Unacceptable, W = Waived, I = Invalid

EPA Primary Reviewer: Byron T. Backus, Ph.D.
Technical Review Branch, Registration Division (7505P)
EPA Secondary Reviewer: Masih Hashim, D.V.M., Ph.D.
Technical Review Branch, Registration Division (7505P)

Signature: Byron T. Backus
Date: 9/6/2007
Signature: Masih Hashim
Date: 9/6/07

DATA EVALUATION RECORD

STUDY TYPE: Companion Animal Safety - Kittens (OPPTS 870.7200)

PC CODES: [129099 (Imidacloprid); 129032 (Pyriproxyfen)] – Not tested in this study

DP BARCODE: D338710

DECISION NO.: 215319

RISK MANAGER: (EPA): 01

TEST MATERIAL AND PRODUCT: The proposed product contains Imidacloprid (9.1%) and Pyriproxyfen (0.46%), as well as one or more solvents. What was tested in this study was the formulation without active ingredients, but with added water (4.6% of the resulting formulation).

CITATION: Abraham, A. S. (2000). Evaluation of the General Safety of 9.1% Imidacloprid with 0.46% (W/W)% Pyriproxyfen Spot-on with 4.6% (W/W) Water Blank Formulation at Three Times the Use Rate Volume in the Target Species, 8-Week Old Kittens. Performing Laboratory: Bayer Corporation Agricultural Division Animal Health DeSoto Research Facility, DeSoto, Kansas 66018. Laboratory Project ID 75191 (150.937). Study Completion Date: October 19, 2000. MRID 47089405. 133 p. + a 2 p. confidential appendix.

SPONSOR: Bayer Corporation Agriculture Division

SUBMITTER: Bayer HealthCare LLC, Animal Health Division, P.O. Box 390, Shawnee, KS 66201

EXECUTIVE SUMMARY: In a companion animal safety study (MRID 47089405), there were two groups, each containing 7 male and 7 female kittens (from 7 weeks 5 days to 8 weeks old at first dosing; day -1 bodyweights: males: 1.48-2.14 lbs; females: 1.36-1.92 lbs; source: Liberty Research Inc., Waverly, NY). Kittens in Group A were treated with the proposed formulation without the actives (but with 4.6% added water) at 3X the label exposure rate for solvents (3 x [0.4 – 0.04] mL = ~1.1 mL; this does not correct for the added water) while kittens in Group B received no treatment and served as controls.

The dose was applied topically on the backside of the head and the neck of each kitten to avoid runoff. Kittens were treated on Days 0, 7, 14 and 21; the proposed label indicates once-a-month treatment, so that each of the kittens in Group A received a cumulative total of 12X of the proposed monthly dosage of the formulation solvents. Kittens in Group B received no exposure to any test material. On Day 13 the heaviest kitten in Group A weighed 2.46 lbs, so none had reached a weight >9 lbs (4.1 kg) which would have resulted in an increase in the 1X dose from 0.36 to 0.72 mL (Day 13 weights were used to set the dosages for Days 14 and 21).

On the days of dosing (Days 0, 7, 14 and 21) each kitten was observed five times, once prior to dosage and then at hourly intervals for four hours after application. Otherwise, clinical observations were made twice a day. Individual daily food consumption was determined visually, using a scoring system ($\geq$75% consumption = 1, 25-75% consumption = 2, $\leq$25% consumption = 3). The kittens

were weighed six times before and during the study (Days -14, -7, -1, 13, 28 and 35). Blood samples were taken on Days -7, -1, 1, 22, and 35. On all collection days, some kittens were rebled due to clotting of the initial sample. Prothrombin time and activated partial thromboplastic time measurements were not done because of the comparatively large amount of blood required for these tests and the age of the kittens; this protocol deviation had been previously discussed with and accepted by the Agency.

There were no mortalities, as all kittens survived to the termination of the study on Day 35. One Group A male (#893) showed signs (unsteadiness, tremors) of toxicity on Days 1-3; this kitten also showed significant decreases in phosphorus and calcium (41.9% and 40% respectively) on Day 1 from Day -1 levels. Kitten #893 also showed an increase in neutrophil count on Day 1 (Day -1: 11.09 x 10^3/µL; Day 1: 18.94 x 10^3/µL). Kitten #893 had no littermates in either Group A or B.

Two additional Group A kittens (#871 & #882) showed slight – but noticeable – decreases in phosphorus and calcium on Day 1 (#871: 27.8% and 8.5% respectively from Day -1 values; #882: 25.8% and 11.7% respectively from Day -1 values), and kitten #871 (but not #882) also showed an increase in neutrophil count on Day 1 (Day -1: 3.43 x 10^3/µL; Day 1: 12.68 x 10^3/µL).

A number of kittens (6 in Group A and 4 in Group B) had soft (loose) stools and/or diarrhea in the period from 1 to 6 days after the first dosage.

Following treatment on Day 7 Group A kitten #882 showed depression, slowness and dehydration. However, this kitten had diarrhea on Days 5 through 7; following the day 7 treatment #882 continued to show soft stools/diarrhea through Day 20. This kitten was treated for diarrhea and dehydration on days 7 through 9. These effects were not considered to be related to exposure to the test material.

Group A kittens all showed a rough hair coat (localized effect?) following applications on Days 0, 7, 14 and 21. This is not considered a toxic effect.

This study is classified as **Supplementary** as a companion animal safety study (OPPTS 870.7200) in 8 week-old kittens, in part because it did not involve actual testing of the proposed formulation with actives. This study was apparently conducted (at least in part) to establish the existence of a 3X safety factor with respect to the normal use application exposure levels of the solvent(s) of the proposed Imidacloprid-Pyriproxyfen formulation and the level at which toxicity occurs. As one of the 14 Group A kittens showed signs of toxicity (including unsteadiness and tremors) on Days 1-3 similar to those observed in kittens exposed to 5.6X or 5X levels of the solvent(s) in other studies (MRIDs 47089401 and 47089403) a 3X margin of safety was not established.

COMPLIANCE: Signed and dated Quality Assurance (p. 4), [No] Data Confidentiality (p. 2) and Good Laboratory Practice Compliance (p. 3) statements are present.

I. MATERIAL

MATERIALS

1. Test material: The test material without the 2 active ingredients (9.1% Imidacloprid and 0.46% Pyriproxyfen), but with 4.6% added water.
   - Description: A liquid, specific gravity not reported.
   - Lot No.: 99-901-101.
   - Storage: Stored in amber glass bottles at room temperature.
   - Placebo: None (controls were not exposed to any test substance)
   - Description: N/A
   - Lot No.: N/A
   - Storage: N/A

2. Administration: Topically applied to the backside of the head and the neck of each kitten to avoid run off.
3. Test animals
   Species: Cat
   Breed: Domestic Short hair
   Ages and weights at study initiation (Day 0 for ages, Day -1 for weights): 7 weeks 5 days to 8 weeks; males: 1.48 to 2.14 lbs; females: 1.36 to 1.92 lbs.
   Source: Liberty Research Inc., Waverly, NY
   Vaccinations and other medications: The kittens had been vaccinated with a four way feline vaccine, Fel-O-Vax® IV (Feline Rhinotracheitis, Calici-Panleukopenia-Chlamydia Psittaci Vaccine Killed Virus and Chlamydia) on Study Days -14 and --1. During the course of the study one kitten (#882) in Group A was treated for diarrhea and dehydration, as were two kittens (#883, #884) in Group B.
   Housing: individual in cages with approximately 3.3 ft^2 of floor space per cage.
   Diet: Harlan Teklad® (commercial dry cat feed) and a canned kitten food (Feline Growth) from Hill's Pet Nutrition, Kansas City, MO. (fed once daily, however, no information is provided as to the amount that was offered).
   Water: Tap water, *ad libitum*
   Environmental conditions:
      Temperature: (not stated)
      Humidity: (not stated)
      Air changes: (not stated)
      Photoperiod: 9-14 hours of lighting/day
   Acclimation period: 14 days

II. STUDY DESIGN

A. IN LIFE DATES

From the report (p. 12 of MRID 47089403) Day 0 was January 13, 2000. The experimental phase of the study was completed on February 17, 2000.

B. ANIMAL ASSIGNMENT/ DOSAGE AND ADMINISTRATION

From p. 12 of MRID 47089405: "Twenty-eight kittens were randomly allocated to two groups. Animals were blocked by sex and ranked by ascending order of study day -1 body weight, and assigned a random number. From the first block (female), the animal with the larger of the first two random numbers was assigned to Group A (vehicle), and the smaller to Group B (no treatment) and so forth until all the animals in the same sex were assigned. This procedure was repeated for the males..."

From p. 16 of MRID 47089405: "The volume of product that is active ingredients is approximately 10%. Therefore, for this study, the normal dose for a kitten less than 9 lbs would be 0.36 mL (90% of 0.4). Based on this product design, the exaggerated doses were based on dose volumes and a kitten weighing less than 9 lbs would receive (3 times 0.36 mL) 1.1 mL volume for each treatment."

From p. 17 of MRID 47089405: "Seven male and seven female kittens in Group A were dosed with an equivalent volume of vehicle at 3X the monthly use rate volume of a 9.1% imidacloprid with 0.46% pyriproxyfen formulation minus the active ingredients (1.1 mL / kitten weighing between 0 and 9 lbs)... Seven male and seven female kittens in Group B served as negative controls and were not treated."

From p. 17 of MRID 47089405: "The dose was administered topically on the back of the head and neck to avoid dose run off of the vehicle. of each kitten [in Group A] to avoid run off. The kittens [in Group A] were dosed four times, on study days 0, 7, 14, and 21."

TABLE 1. Study design

Group & Weight Range (lb)		Number of kittens	Mean Kitten Weight: Mean Kitten Wt ± S.D. (lb) on Day -1 (before 1st application)	Mean Kitten Wt ± S.D. (lb) on Day 13 (before 3rd application)	Mean Kitten Wt ± S.D. (lb) on Study Day 28	Mean Kitten Wt ± S.D. (lb) on Study Day 35
(A): 5X solvent	males ≤ 9 lb	7	1.77 ± 0.17	2.19 ± 0.23	2.81 ± 0.20	3.25 ± 0.24
	females ≤ 9 lb	7	1.60 ± 0.14	1.91 ± 0.21	2.51 ± 0.21	2.90 ± 0.21
	combined ≤ 9 lb	14	1.69 ± 0.17	2.05 ± 0.26	2.66 ± 0.25	3.08 ± 0.28
(B): no treatment	males ≤ 9 lb	7	1.75 ± 0.22	2.19 ± 0.18	2.81 ± 0.19	3.26 ± 0.17
	females ≤ 9 lb	7	1.66 ± 0.16	2.01 ± 0.30	2.58 ± 0.28	2.99 ± 0.28
	combined ≤ 9 lb	14	1.71 ± 0.19	2.10 ± 0.26	2.70 ± 0.26	3.13 ± 0.27

Data calculated from information on p. 28 and 29 of MRID 47089405.

C. DOSE SELECTION RATIONALE

From p. 9 of MRID 47089405: "This companion animal safety study was conducted to evaluate the general safety of 9.1% Imidacloprid with 0.46% Pyriproxyfen (W/W) with 4.6% water blank formulation on kittens applied at 3X use volume of the inert ingredients at weekly intervals for a total of 4 treatments in 8-week old kittens..."

D. EXPERIMENTAL DESIGN

There were two groups, each containing 7 male and 7 female kittens (from 7 weeks 5 days to 8 weeks old; day -1 bodyweights: males: 1.48-2.14 lbs; females: 1.36-1.92 lbs). Kittens in Group A were treated with the proposed product without the actives (and with 4.6% added 0water) at 3X the label-specified use exposure rate for solvents (3 x [0.4-0.04 mL] ~ 1.1 mL) while kittens in Group B were not treated with anything. The dose was applied topically on the backside of the head and the neck of each kitten in Group A to avoid runoff. The dose was administered by parting the hair and using a syringe without a needle. Kittens were treated on Days 0, 7, 14 and 21; since the proposed label indicates once-a-month treatment, each of the kittens in Group A received a cumulative total of 12X of the proposed monthly dosage of the formulation solvents. Group B kittens served as negative controls.

At the last weighing (Day 35) the heaviest kitten weighed 3.46 lbs, so none had reached a weight >9 lbs (4.1 kg) which would have resulted in an increase in the 3X dose from 1.2 to 2.4 mL.

On the days of dosing (Days 0, 7, 14 and 21) each kitten was observed five times, once prior to dosage and then at hourly intervals for four hours after application. Otherwise, clinical observations were made twice (once in the a.m., once in the p.m.) a day. Individual daily food consumption was determined visually, using a scoring system (≥75% consumption = 1, 25-75% consumption = 2, ≤25% consumption = 3). The kittens were weighed at six times before and during the study (Days -14, -7, -1, 13, 28 and 35).

Blood samples were taken on Days -7, -1, 1, 22, and 35. It was necessary to obtain additional blood samples from individual kittens on several days due to insufficient quantities initially obtained or because of blood sample clotting. Prothrombin time and activated partial thromboplastic time measurements were not done because of the comparatively large amount of blood required for these tests and the age of the kittens; this protocol deviation had been previously discussed with and accepted by the Agency.

From p. 18 of MRID 47089405: "Physical examinations were performed on the study animals on study days -10, -1, and 35."

E. CLINICAL PATHOLOGY PARAMETERS

Blood samples were collected from each kitten on study Days -7, -1, 1, 22 and 35. There is no indication within the report that kittens were fasted prior to collection of blood. The CHECKED (X) parameters were examined:

a. Hematology

X		X	
X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Absolute and percent basophil count
		X	Absolute and percent eosinophil count
X	Leukocyte count (WBC)*	X	Absolute and percent lymphocyte count
X	Erythrocyte count (RBC)*	X	Absolute and percent monocyte count
		X	Absolute and percent neutrophil count
X	Platelet count (PLTS)	X	Mean corpuscular HGB (MCH)*
	Blood clotting measurements	X	Mean corpusc. HGB conc.(MCHC)*
	(Thromboplastin time)	X	Mean corpusc. volume (MCV)*
	(Clotting time)		
	(Prothrombin time [PT])*		
	(Activated partial thromboplastin time [APTT])*		

*Recommended in OPPTS 870.7200 Guidelines. The Prothrombin time and Activated partial thromoplastin time were not done because of the comparatively large volume of blood required for these tests and the age of the kittens. This deviation had been accepted by the Agency prior to initiation of this study.

b. Clinical chemistry

X	ELECTROLYTES	X	OTHER
X	Calcium*	X	Albumin (Alb)*
X	Chloride*	X	Creatinine (Crea)*
	Magnesium	X	Blood urea nitrogen (BUN)*
X	Phosphorus*		Total Cholesterol
X	Potassium*	X	Globulin (Glob)*
X	Sodium*	X	Glucose (Gluc)*
		X	Total bilirubin (T Bil)*
	ENZYMES	X	Direct bilirubin (D Bil)*
			Total protein (TP)*
X	Alkaline phosphatase(ALP or ALK)*		Triglycerides
	Cholinesterase(ChE)		Serum protein electrophoresis
	Creatine phosphokinase	X	Albumin/Globulin (A/G) ratio
	Lactic acid dehydrogenase(LDH)		Lipase
X	Serum alanine aminotransferase (ALT or SGPT)*		
X	Serum aspartate aminotransferase(AST or SGOT)*	X	BUN/Creatinine ratio
		X	Ca/Phos Ratio
	Gamma glutamyl transpeptidase(GGT)	X	Na/K Ratio
	Amylase		

*Recommended in OPPTS 870.7200 Guidelines.

F. CONCOMITANT MEDICATIONS/THERAPIES

From p. 17 of MRID 47089405: "One kitten with diarrhea and suspected to have bacterial (negative for coccidian) enteritis was treated subcutaneously with Excenel at 1 mg per day for two days (study day -9 and -8). On study day 7 to 9, one kitten (#882 – group A) was treated for diarrhea and dehydration. Kitten #882 was treated subcutaneously with Excenel at 2 mg per day and 12 mL of Lactated Ringers Solution (Sodium Chloride 600 mg, Sodium Lactate 310 mg, Potassium Chloride 30 mg, Calcium Chloride Dihydrate 20 mg and Water for Injection q.s. per 100 mL) twice a day except one treatment on study day 9. On study day 7 and 8 one kitten (#883 – group B) was treated for diarrhea (negative for coccidian). Kitten #883 was treated subcutaneously with Excenel at 2 mg per day. On study day 13 and 14 one kitten (#884 – group B) was treated for diarrhea and dehydration. Kitten #884 was treated once a day subcutaneously with Excenel at 2 mg and 12 mL of Lactated Ringers Solution."

G. STATISTICS

Although means and standard deviations were calculated for some parameters (such as body weight), statistical tests were primarily applied to chemistry and hematology pathology parameters. From p. 113 of MRID 47089405: "This study is intended to confirm the general safety of the vehicle and approximately the same number of adverse effects is expected between the two groups. Adverse effects will be summarized in tables. Certain types of adverse effects may be grouped together, depending on the clinical presentation, such as all effects, all transient effects or all blood chemistry

effects. If the number or pattern of effects elicit clinical interest, incidence rates will also be compared between groups." For the clinical pathology parameters, it is stated (p. 19 of MRID 47089405): "For each animal, a baseline value was calculated for each clinical pathology test, by averaging the two pretreatment measurements (study days -7 and -1). Each clinical pathology test was then analyzed with a multivariate repeated measures ANOVA (baseline, study days 1, 22, and 35) including terms for Group, Sex, Animal (random), Day, and Group*Day as the predictors..."

H. DISPOSITION OF ANIMALS

From p. 12 of MRID 47089405: "One animal not included in the experimental phase of the study was euthanized due to poor health on December 30, 1999 (study day -14). One animal not included in the experimental phase of the study was euthanized due to poor health on January 03, 2000 (study day -10). One kitten was found dead on study day -10. The cause of death was due to dehydration and lack of nutrients due to enteritis of undetermined origin. Thirteen animals not included in the experimental phase of the study were euthanized on January 13, 2000 (study day 0). The remaining 28 animals included in the experimental phase of the study were not euthanized at the end of the study (February 17, 2000)." According to the OPPTS 870.7200 Guidelines: "Routine sacrifice or necropsy is not required for surviving animals."

I. COMPLIANCE

Signed and dated Quality Assurance [p. 4], [No] Data Confidentiality [p. 2], and Good Laboratory Practice (GLP) Compliance [p. 3] Statements were present.

III. RESULTS

A. EXPOSURE LEVELS

Refer to Table 1 of this DER. Kittens in the control group (Group B), all weighing $\leq$ 9.0 lb, were not dosed, while kittens in the test group (Group A), also all weighing $\leq$ 9.0 kg, were dosed with 1.1 mL of the formulation without actives (but with 4.6% added water) at each application. Applications were made on Days 0, 7, 14 and 21.

B. MORTALITY

There was no mortality. All kittens survived the 35-day observation period.

C. CLINICAL SIGNS

Group A: One male (#893) showed signs (unsteadiness, tremors, "proprioception deficit") of toxicity on Days 1-3, consistent with the signs observed in some kittens exposed to 5.6X and 5X in other studies (MRIDs 47089401 & 47089403). However, male #893 showed no signs of toxicity on Day 4 or subsequently (including after applications on Days 7, 14 and 21). Male #893 consumed <25% of the food that was offered on Day 2.

TABLE 2a. Observed Signs of Toxicity After First Application (Day 0)

Group	Signs in the 4-hour observation period	Signs in the 1-3 days following treatment
5X Vehicle (A)	All treated kittens with rough hair coat following application except for males #886 and #893. One male (#886) and one female (#882) had soft (loose) stools before application.	Male #893 showed unsteadiness, tremors, "proprioception deficit." Five kittens (females #863, 882; males #864, #886, #888) showed soft (loose) stools or diarrhea.
No Treatment (B)	Diarrhea or soft stools in females #866 and #883.	Three kittens (females #866, #883, and #884) with loose (soft) stools and/or diarrhea on days 1 and/or 2 and/or 3.

Data from information on p. 32 of MRID 47089405.

TABLE 2b. Observed Signs of Toxicity After Second Application (Day 7)

Group	Signs in the 4-hour observation period	Signs in the 1-3 days following treatment
5X Vehicle (A)	All treated kittens with rough hair coat following application. One female (#882) with diarrhea, depression, slowness & dehydration prior to and after application. One male (#864) with soft stools prior to application.	Female #882: depression, dehydration and slowness on day 8; soft stools on day 9. One female (#860) with soft stools on day 9.
No Treatment (B)	Two females (#866, #883) with diarrhea and/or soft stools.	One female (#883) with soft stools on day 8.

Data from information on p. 33 of MRID 47089405.

TABLE 2c. Observed Signs of Toxicity After Third Application (Day 14)		
Group	**Signs in the 4-hour observation period**	**Signs in the 1-3 days following treatment**
5X Vehicle (A)	All treated kittens with rough hair coat following application. One female (#882) with diarrhea prior to application; one male (#886) with soft stools prior to application.	Female #882 with diarrhea or soft stools; male #886 with soft stools.
No Treatment (B)	Female #884 with slowness (this female had diarrhea, depression & slowness on day 13).	Female #884 & male #867 with soft stools.

Data from information on p. 33-34 of MRID 47089405.

TABLE 2d. Observed Signs of Toxicity After Fourth Application (Day 21)		
Group	**Signs in the 4-hour observation period**	**Signs in the 1-3 days following treatment**
5X Vehicle (A)	All treated kittens with rough hair coat following application.	Female #861 vomited on day 22.
No Treatment (B)	None.	Male #869 vomited on day 22.

Data from information on p. 34-35 of MRID 47089405.

D. NEUROLOGICAL OBSERVATIONS

The effects (unsteadiness, tremors) observed in Group A male #893 were consistent with neurotoxicity, and with the signs seen in some kittens exposed to 5.6X and 5X the same formulation solvents/inerts in other studies (MRIDs 47089401 & 47089403).

E. BODY WEIGHT AND WEIGHT GAIN

Most kittens (in both groups) had good weight gains from day -1 to 13, from day 13 to 28, and again from day 28 to 35. One Group B kitten (female #884) had a slight weight loss in the period from Day -1 to 13 (from 1.44 lbs on Day -1 to 1.41 lbs on Day 13). This kitten had a number of occurrences of diarrhea or soft stools, and was dehydrated on Day 13. This kitten also consumed <25% of the offered food on Days 0 and 13 (these represented 2/3 of the occurrences of this among the 28 kittens in the study in the period from Day -12 to 35, the other being in Group A male #893 on Day 2).

F. FOOD CONSUMPTION

No information is provided as to the exact (or even approximate) amount of food that was offered to kittens on a daily basis. As agreed with EPA, the amount of food consumed was determined visually, with 1 representing $\geq$ 75% offered food consumed, 2 being 25-75% consumption, and 3 representing <25% consumption. Most of the food consumption values were "1," particularly after Day 14. The only value of "3" in Group A (vehicle treated kittens) after Day 0 was with male #893 on Day 2 (when this kitten was showing signs of toxicity). Four Group A kittens (females #871, #880 and #882, and

male #893) had values of "2" on day 1. None of the Group B kittens had any food consumption value other than "1" for Day 1, but two (female #866 & male #887) had a value of "2" on Day 2.

G. HEMATOLOGY

Group A Kitten #893 (signs of toxicity on Days 1-3) had an increase in neutrophil count on Day 1 (Day -1: 11.09 x 10^3/µL; Day 1: 18.94 x 10^3/µL). Group A kitten #871 (but not #882) also showed an increase in neutrophil count on Day 1 (Day -1: 3.43 x 10^3/µL; Day 1: 12.68 x 10^3/µL).

H. CLINICAL CHEMISTRY

Group A kitten #893 (signs of toxicity on Days 1-3) showed significant decreases in phosphorus and calcium (41.9% and 40% respectively) on Day 1 from Day -1 levels. Two additional Group A kittens (#871 & #882) showed slight – but noticeable – decreases in phosphorus and calcium on Day 1 (#871: 27.8% and 8.5% respectively from Day -1 values; #882: 25.8% and 11.7% respectively from Day -1 values), and phosphatase (ALP), and decreases in potassium (K), phosphorus (P) and calcium (Ca).

TABLE 3a. Selected Hematology and Blood Chemistry Values for #893

Kitten + Parameter	Day -7	Day -1	Day 1	Day 22	Day 35
#893:					
Neutrophils	8.60 x10^3/µL	11.09x10^3/µL	**18.94x10^3**/µL	6.31 x 10^3/µL	4.44 x 10^3/µL
Glucose	127 mg/dL	119 mg/dL	130 mg/dL	120 mg/dL	106 mg/dL
Potassium (K)	5.7 mmol/L	6.5 mmol/L	5.6 mmol/L	7.1 mmol/L	5.0 mmol/L
Phosphorus (P)	7.0 mg/dL	7.4 mg/dL	**4.3** mg/dL	8.4 mg/dL	7.3 mg/dL
Calcium (Ca)	10.7 mg/dL	11.5 mg/dL	**6.9** mg/dL	10.9 mg/dL	10.4 mg/dL
ALP	85 u/L	98 u/L	**51** u/L	124 u/L	104 u/L
AST	21 u/L	29 u/L	25 u/L	30 u/L	18 u/L
ALT	26 u/L	36 u/L	33 u/L	44 u/L	42 u/L

Data from information on p. 81 of MRID 47089405.

Group A female #882, with no signs of toxicity following Day 0 exposure, did have slight decreases in potassium, phosphorus, calcium and alkaline phosphatase activity on Day 1, but there was no biologically significant change in any of the other parameters.

TABLE 3b. Selected Hematology and Blood Chemistry Values for #882

Kitten + Parameter	Day -7	Day -1	Day 1	Day 22	Day 35
#882:					
Neutrophils	15.83 x10^3/µL	11.33x10^3/µL	8.35 x10^3/µL	7.84 x 10^3/µL	8.44 x 10^3/µL
Glucose	123 mg/dL	128 mg/dL	103 mg/dL	136 mg/dL	112 mg/dL
Potassium (K)	5.5 mmol/L	5.9 mmol/L	**4.5** mmol/L	5.0 mmol/L	5.4 mmol/L
Phosphorus (P)	9.9 mg/dL	9.7 mg/dL	**7.2** mg/dL	9.0 mg/dL	8.9 mg/dL
Calcium (Ca)	10.9 mg/dL	11.1 mg/dL	**9.8** mg/dL	10.4 mg/dL	10.3 mg/dL
ALP	94 u/L	110 u/L	**59** u/L	119 u/L	113 u/L
AST	25 u/L	23 u/L	30 u/L	28 u/L	23 u/L

ALT	39 u/L	74 u/L	78 u/L	43 u/L	46 u/L

Data from information on p. 73 of MRID 47089405.

Group A female #871, with no signs of toxicity following Day 0 exposure, did have slight decreases in potassium, phosphorus, calcium and alkaline phosphatase activity on Day 1, along with an increase in neutrophil count, but there was no biologically significant change in any of the other parameters.

TABLE 3b. Selected Hematology and Blood Chemistry Values for #871

Kitten + Parameter	Day -7	Day -1	Day 1	Day 22	Day 35
#871:					
Neutrophils	4.20 x10^3/µL	3.43 x10^3/µL	**12.68** x10^3/µL	4.58 x 10^3/µL	3.92 x 10^3/µL
Glucose	128 mg/dL	120 mg/dL	115 mg/dL	111 mg/dL	110 mg/dL
Potassium (K)	5.2 mmol/L	5.6 mmol/L	**4.3** mmol/L	4.5 mmol/L	5.9 mmol/L
Phosphorus (P)	6.5 mg/dL	7.6 mg/dL	**5.5** mg/dL	7.1 mg/dL	7.1 mg/dL
Calcium (Ca)	10.3 mg/dL	10.7 mg/dL	**9.8** mg/dL	10.7 mg/dL	10.5 mg/dL
ALP	136 u/L	148 u/L	**54** u/L	216 u/L	173 u/L
AST	22 u/L	24 u/L	26 u/L	24 u/L	19 u/L
ALT	26 u/L	40 u/L	28 u/L	44 u/L	44 u/L

Data from information on p. 67 of MRID 47089405.

I. NECROPSY FINDINGS

As there were no deaths, there were no necropsies.

IV. DISCUSSION

In a companion animal safety study (MRID 47089405), there were two groups, each containing 7 male and 7 female kittens (from 7 weeks 5 days to 8 weeks old at first dosing; day -1 bodyweights: males: 1.48-2.14 lbs; females: 1.36-1.92 lbs; source: Liberty Research Inc., Waverly, NY). Kittens in Group A were treated with the proposed formulation without the actives (but with 4.6% added water) at 3X the label exposure rate for solvents (3 x [0.4 – 0.04] mL = ~1.1 mL; this does not correct for the added water) while kittens in Group B received no treatment and served as controls.

The dose was applied topically on the backside of the head and the neck of each kitten to avoid runoff. Kittens were treated on Days 0, 7, 14 and 21; the proposed label indicates once-a-month treatment, so that each of the kittens in Group A received a cumulative total of 12X of the proposed monthly dosage of the formulation solvents. Kittens in Group B received no exposure to any test material. On Day 13 the heaviest kitten in Group A weighed 2.46 lbs, so none had reached a weight >9 lbs (4.1 kg) which would have resulted in an increase in the 1X dose from 0.36 to 0.72 mL (Day 13 weights were used to set the dosages for Days 14 and 21).

On the days of dosing (Days 0, 7, 14 and 21) each kitten was observed five times, once prior to dosage and then at hourly intervals for four hours after application. Otherwise, clinical observations were made twice a day. Individual daily food consumption was determined visually, using a scoring system (≥75% consumption = 1, 25-75% consumption = 2, ≤25% consumption = 3). The kittens were weighed six times before and during the study (Days -14, -7, -1, 13, 28 and 35). Blood samples were taken on Days -7, -1, 1, 22, and 35. On all collection days, some kittens were rebled due to clotting of the initial sample. Prothrombin time and activated partial thromboplastic time measurements were not done because of the comparatively large amount of blood required for these tests and the age of the kittens; this protocol deviation had been previously discussed with and accepted by the Agency.

There were no mortalities, as all kittens survived to the termination of the study on Day 35. One Group A male (#893) showed signs (unsteadiness, tremors) of toxicity on Days 1-3; this kitten also showed significant decreases in phosphorus and calcium (41.9% and 40% respectively) on Day 1 from Day -1 levels. Kitten #893 also showed an increase in neutrophil count on Day 1 (Day -1: 11.09 x 10^3/µL; Day 1: 18.94 x 10^3/µL). Kitten #893 had no littermates in either Group A or B.

Two additional Group A kittens (#871 & #882) showed slight – but noticeable – decreases in phosphorus and calcium on Day 1 (#871: 27.8% and 8.5% respectively from Day -1 values; #882: 25.8% and 11.7% respectively from Day -1 values), and kitten #871 (but not #882) also showed an increase in neutrophil count on Day 1 (Day -1: 3.43 x 10^3/µL; Day 1: 12.68 x 10^3/µL).

A number of kittens (6 in Group A and 4 in Group B) had soft (loose) stools and/or diarrhea in the period from 1 to 6 days after the first dosage.

Following treatment on Day 7 Group A kitten #882 showed depression, slowness and dehydration. However, this kitten had diarrhea on Days 5 through 7; following the day 7 treatment #882 continued to show soft stools/diarrhea through Day 20. This kitten was treated for diarrhea and dehydration on days 7 through 9. These effects were not considered to be related to exposure to the test material.

Group A kittens all showed a rough hair coat (localized effect?) following applications on Days 0, 7, 14 and 21. This is not considered a toxic effect.

This study is classified as **Supplementary** as a companion animal safety study (OPPTS 870.7200) in 8 week-old kittens, in part because it did not involve actual testing of the proposed formulation with actives. This study was apparently conducted (at least in part) to establish the existence of a 3X safety factor with respect to the normal use application exposure levels of the solvent(s) of the proposed Imidacloprid-Pyriproxyfen formulation and the level at which toxicity occurs. As one of the 14 Group A kittens showed signs of toxicity (including unsteadiness and tremors) on Days 1-3 similar to those observed in kittens exposed to 5.6X or 5X levels of the solvent(s) in other studies (MRIDs 47089401 and 47089403) a 3X margin of safety was not established.

ACUTE TOX ONE-LINERS

1. **DP BARCODE:** D338710
2. **PC CODES:** N/A (solvents only tested)
3. **CURRENT DATE:** 5 September 2007
4. **TEST MATERIAL:** The proposed product contains Imidacloprid (9.1%) and Pyriproxyfen (0.46%), as well as one or more solvents. What was tested in this study was the formulation (solvent[s] + inerts) without active ingredients, but with added water (4.6% of the resulting formulation).

Study/Species/Lab Study # /Date	MRID	Results	Tox. Cat.	Core Grade
Companion animal safety / 8-week old kitten / Intervet Inc, DeSoto, KS / Project ID 75191(150.937) / 19-OCT-2000	47089405	2 groups, each containing 7M & 7F 8-week old domestic short hair kittens were used. Group A was treated at 3X with the solvents and inerts (no actives) of the proposed formulation on days 0, 7, 14 & 21 while Group B was untreated. All kittens survived the 35-day observation period, although one male showed signs (unsteadiness, tremors) of toxicity on Days 1-3. This kitten showed significant decreases in phosphorus and calcium (41.9% & 40% respectively) on Day 1 from Day -1 levels; also an increase in neutrophil count (Day -1: 11.09 x $10^3/\mu L$; Day 1: 18.94 x $10^3/\mu L$); two additional Group A kittens without symptoms also showed slight decreases in phosphorus & calcium on Day 1, and one showed an increase in neutrophil count. Study does not demonstrate a 3X safety factor for the proposed product.	N/A	S

Core Grade Key: A =Acceptable, S = Supplementary, U = Unacceptable, W = Waived, I = Invalid